FAO Fisheries Technical Paper No. 175 FIR/T175

STANDARD TECHNIQUES FOR PELAGIC FISH EGG AND LARVA SURVEYS

APR 24 1975

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

STANDARD TECHNIQUES FOR PELAGIC FISH EGG AND LARVA SURVEYS

by

Paul E. Smith
Southwest Fisheries Center
NMFS, NOAA, US Dept. of Commerce
La Jolla, California 92038, USA

and

Sally L. Richardson
Department of Oceanography
Oregon State University
Corvallis, Oregon 97331, USA

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, December 1977

The designations employed and the presentation of material in this publication do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

M-43
ISBN 92-5-100515-X

The copyright in this book is vested in the Food and Agriculture Organization of the United Nations. The book may not be reproduced, in whole or in part, by any method or process, without written permission from the copyright holder. Applications for such permission, with a statement of the purpose and extent of the reproduction desired, should be addressed to the Director, Publications Division, Food and Agriculture Organization of the United Nations, Via delle Terme di Caracalla, 00100 Rome, Italy.

© FAO 1977

PREPARATION OF THIS PAPER

This publication is one of a series of FAO manuals in fisheries science. It has been prepared as part of the programme of the Fishery Resources and Environment Division and is largely based on the work of a Working Party on Fish Egg and Larval Surveys established following the recommendation of the Fourth and Fifth Sessions of the FAO Advisory Committee on Marine Resources Research.

Through a contract awarded to the Southwest Fisheries Center, National Marine Fisheries Service, La Jolla, California, the present version of the manual was prepared by Paul E. Smith and Sally L. Richardson.

The manual will be primarily for use in FAO field projects and training courses, and by national institutions, especially in developing countries, which may be conducting fish egg and larval survey programmes. The final edition of the paper in the official languages of FAO will eventually be published incorporating any revisions that may be proposed by the users of the manual.

Distribution

FAO Department of Fisheries
FAO Regional Fishery Officers
Selector SM
UNESCO
IOC
SCOR
Author

Bibliographic reference

Smith, P.E. & S. Richardson (1977) FAO Fish.Tech.Pap., (175):100 p. Standard techniques for pelagic fish egg and larval surveys

Ichthyoplankton surveys. Methodology. Sampling (biological). Plankton collecting devices. Stock assessment.

PREFACE

A Working Party on Fish Egg and Larval Surveys was established in 1968 following the recommendation of the fourth and fifth sessions of the FAO Advisory Committee on Marine Resources Research (ACMRR), whose terms of reference included the preparation of a Manual (field guide) for this aspect of fishery science. The contributions prepared by the Working Party members mainly based on the discussions of the Working Party held in Tunis from 22 to 27 October 1969 and on further advice provided by ACMRR during its Sixth Session, were recently edited by Prof. G. Hempel, and published by FAO: Fish Egg and Larval Surveys (Contributions to a Manual) (FAO Fish.Tech.Pap., 122). This provisional version has been produced for use as a background document during the first International Training Course on Fish Egg and Larval Studies, held in La Jolla from 2 to 29 September 1973 (see FAO.Fish. Rep., 144). Based on the experience gained during the course and taking into account the recommendation of the ACMRR that the Manual should provide a review of the circumstances under which egg and larval surveys are more appropriate and cost-effective than other field surveys, the provisional version was edited and supplemented in order to provide the potential new users of this survey technique with a balanced view of its value and limitations as well as of cost effectiveness.

Many people have been generous with their time and thought to make this manual possible. The consistent support and encouragement of Dr. Elda Fagetti has been the essential energy behind this work and the advice and urging of Dr. Gotthilf Hempel and Dr. Elbert Ahlstrom have guided the production of the manual. The authors of a precursor to this manual (Hempel, 1973), E.H. Ahlstrom, J.M. Colebrook, E. Fagetti, G. Hempel, K. Sherman, S. Tanaka have furnished major sections of text within this edition. Much of the procedural information in section 2 has been derived from procedural description (Kramer et al, 1972) relative to the CalCOFI surveys. Significant editing was offered by Dr. William Richards, Dr. William Aron, Dr. William Lenarz, and Mr. David Kramer. Helpful comments were received from Dr. J.D. de Ciechomski, M.E. Diaz, Dr. J.A. Gulland, Dr. J. Kinzer, Dr. R. Lasker, Dr. A. Longhurst, R. Marak, B. Monsalve, Dr. W. Nellen, M.A. Padilla, A.M.Perez-Franco, Dr. B.J. Rothschild, Dr. M. Ruivo, Dr. D. Sahrhage, Dr. H. Santander, Mr. K. Sherman, Dr. A.C. Simpson, Dr. M. Vanucci, and K. Venkataramaniyam. Administrative assistance was received from Dr. E.F. Akyüz, Mr. Ben Remington, Mr. I. Barrett, Mr. Richard Schwartzlose and Ms. Barbara Rowe. Technical assistance was received from K. Akutagawa, F. Crowe, S. HuFei, E. Stevens and J. Thrailkill and typing was by Janica Scott. Dr. Sally Richardson incorporated the comments and reviews, unified the style of the text, and rewrote major sections and she has been named assistant editor for this manual.

Paul E. Smith

CONTENTS

Page

1. Introduction 1
1.1 Background 1
1.2 Scope 5

2. Recommended Procedures for Conducting Ichthyoplankton Surveys for Spawning Biomass Estimates 6
2.1 Field Operations 6
2.1.1 Cruise Plan and Personnel 6
2.1.2 Sampling System 8
2.1.2.1 The Sampler
2.1.2.2 The Towing Procedure and Data Records
2.1.3 Handling the Sample at Sea 16
2.1.3.1 Preserving the Sample
2.1.3.2 Labelling
2.1.3.3 Storage on the Vessel
2.1.4 Supplementary Data 18
2.1.4.1 Hydrographic Data
2.1.4.2 Smaller Zooplankton and Phytoplankton
2.1.4.3 Neuston
2.1.4.4 Micronekton
2.2 Laboratory Procedures 21
2.2.1 Plankton Volume Determinations 21
2.2.2 Percentage of the Sample to be Sorted 23
2.2.3 Sorting Fish Eggs and Larvae from Plankton Samples. 23
2.2.4 Preliminary Identification, Enumeration and Measurement of Fish Eggs and Larvae 26
2.2.5 Final Identification of Fish Eggs and Larvae 27
2.2.6 Bottling, Storing and Curating Identified Collections of Fish Eggs and Larvae 30
2.3 Data Summarization 30
2.3.1 Flowmeter Calibration 34
2.3.2 Estimation of Missing Flowmeter Values 34
2.3.3 Equation of Standardization 36
2.3.4 Data Forms Used for Standardization 36
2.4 Census Estimate (Larval Index) 40
2.5 Estimating the Spawning Biomass 42

3. Theoretical Considerations 43
3.1 Statistics 43
3.1.1 Numbering Systems 43
3.1.1.1 Normal Distribution
3.1.1.2 Lognormal Distribution
3.1.1.3 Negative Binomial Distribution
3.1.2 Data Comparisons 53
3.1.3 Advice on Data Transformations and Comparisons . . . 62
3.2 Volumetric Sampling 63
3.2.1 Volume and Distribution of Water Filtered 63
3.2.1.1 The Oblique Tow
3.2.1.2 Clogging
3.2.2 Avoidance 66
3.2.3 Mesh Retention 69
3.2.4 Uneven Tow Trajectories 73
3.3 Sampling to Determine Spawning Area and Time 74
3.3.1 Geographic Distribution 74
3.3.2 Vertical Distribution 75
3.3.3 Seasonal Distribution 77
3.3.4 Oceanic Transport and Diffusion 80

Page

3.4 Sampling to Overcome Problems of Microdistribution 81
3.5 Residence Time (Estimating Mortality) 83
3.5.1 Temperature Dependent Hatching Time 83
3.5.2 Temperature Dependent Larval Growth Rate 85
3.5.3 Food Dependent Growth Rate 86
3.5.4 Mortality Estimates 86
3.6 Spawning Biomass Estimates 86
3.7 Fecundity 87
3.8 Alternative Quantitative Ichthyoplankton Samplers 88
3.8.1 Oblique Tow Nets with Bridles 89
3.8.2 Vertical Tow Nets with Bridles 89
3.8.3 High-Speed Nets 89
3.9 Intercalibration of Quantitative Samplers 92
3.10 Design of Surveys to Minimize Costs 92

4. References 94

1. Introduction

The purpose of this manual is to describe the techniques necessary for conducting quantitative ichthyoplankton surveys. The main focus of the description will be directed toward the use of such surveys for estimating the spawning biomass of pelagic fishes. However, ichthyoplankton surveys have many other uses (Table 1.1) some of which will also be taken into account within the framework of the text, especially those dealing with detection and appraisal of fishery resources.

1.1. Background

Data on the size of the stock, together with an index of its productivity, are essential to understand the dynamics of an exploited fish stock and to evaluate the effect of fishing on the stock so as to manage the fishery. These indices are usually derived from life history data and statistics of total catch. Various methods have been developed to produce estimates of absolute or relative size of stocks. These methods include:

(i) analysis of catch and effort statistics,

(ii) tagging experiments,

(iii) observation or direct counting,

(iv) sonic surveys,

(v) surveys of eggs and larvae

Under certain conditions, each of the methods may provide a reliable estimate of the size of stocks. There are, however, considerable limitations to the application and accuracy of each of the methods. Therefore, whenever possible, more than one independent estimate should be attempted.

In most cases, much information on stocks is obtained through commercial fisheries which involve various sources of bias. Hence estimates of stock size made independently of the commercial fisheries are very useful. Among the methods listed above, the first two depend much upon commercial fisheries whereas the other three are largely independent of fisheries.

Catch and effort data analysis has been applied to many commercially exploited fish stocks. Whenever possible, this method should be attempted for stock analysis. An advantage of this method is that the basic data for the study are often available without special surveys or with a small amount of additional work. As catch and effort data are needed for many purposes including administrative requirements, surveys for them are conducted as government routine in many countries. Schaefer (1957) and Silliman (1967) provide good examples of catch and effort data analysis with a simple model of population dynamics.

In order to apply a more detailed model, such as that proposed by Beverton and Holt (1957), age specific data (e.g., growth of fish, and age structure in the catch as well as detailed catch and effort statistics classified by areas and seasons) are needed. Collecting these data requires some care but generally the work is not quite as costly as that required for surveys at sea.

One of the most serious limitations of this method is introduced by rapid changes in gear efficiency which are now going on everywhere in the world fisheries. Catch per unit effort will give erroneous patterns of the change in abundance if estimates of major long-term changes in the efficiency of gear are lacking.

Other difficulties include changes in availability and vulnerability related to fish ecology and oceanographic conditions. These changes will seriously affect the landings and catch per unit effort, resulting in imprecise information on the size of the stock and the mortality rates. This is particularly true for swiftly moving pelagic fishes.

Mortality analysis with the catch and effort data is difficult to apply to a fish species with a short life span, or to a stock exploited by several types of gear each specialized for capturing different developmental stages of fish.

Furthermore, detailed and reliable statistics on catch and effort are not always available to fishery scientists and the available data do not necessarily represent the stock because fisheries are often biased by their economic interest.

Tagging fish is a very useful method for estimating abundance and has been used widely for stock analysis (e.g., ICNAF, 1963). In many cases, this method has provided reliable estimates of the size of stock. However, there are many limitations. Differential mortality sometimes makes the application of this method impossible. This is particularly true for small sized fish such as sardine and anchovy or small juveniles. Recovery rate from the catch and reporting rate by fishermen may be considerably lower than unity for the catch treated in mass. Tag shedding and non-random distribution of tagged fish in the stock are among many factors which introduce error and bias in the estimates. Cost of fish for release is sometimes exceedingly high, if a large enough number are to be released for a reliable estimate.

Direct counting by eye can be applied only for large sized fish. The number of schools of fish such as sardine could be counted but this would be performed much better by acoustic instruments.

Table 1.1. On the use of ichthyoplankton surveys. (From Hempel, 1973)

Detection and appraisal of fishery resources
Exploring for new resources
Locating spawning concentrations of important stocks
Describing relative abundances of commercially important stocks; comparison within and amongst regions
Monitoring long-term changes in the composition and abundance of resources and in spawning times and areas
Studies in biology and systematics
Studying the development, growth, behaviour, food requirements and mortality of the early stages of economically important fishes as related to environmental factors
Providing a better understanding of oceanic biology, e.g., zoogeography and ecology of all organisms in the samples
Clarifying fish systematics
Studies in population dynamics of fishes
Tracing fluctuations in spawning stocks by estimating the abundance of their eggs and young larvae
Forecasting year-class strength on the basis of the abundance of older larvae
Estimating abundance of a stock based on its spawning production
Discriminating between stocks of the same species

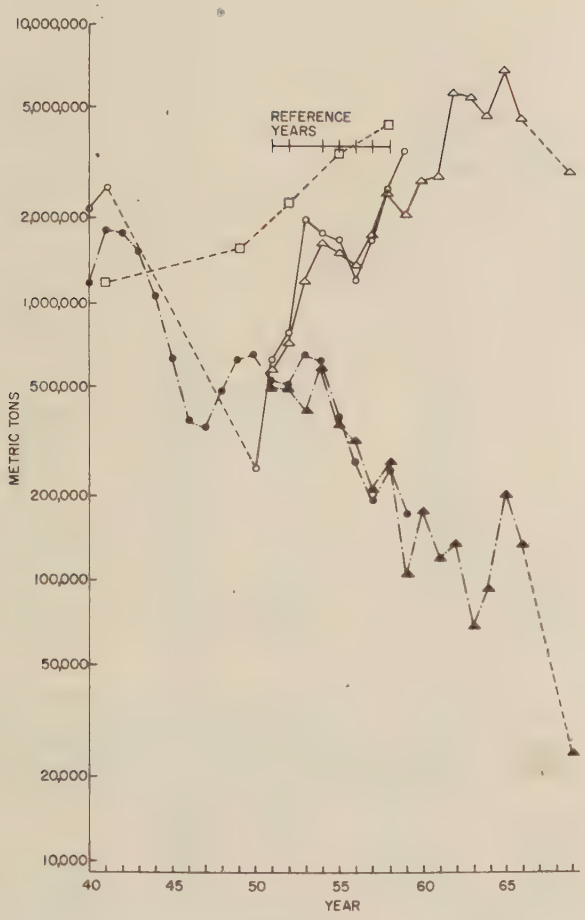


Figure 1.1. A time series comparison of sardine and anchovy biomass estimates from 1940 through 1969. (From Smith, 1972). Dashed lines represent interpolations between non-adjacent years.

● - sardine biomass from 1940 to 1959 calculated from the fishery by Murphy (1966)

▲ - sardine biomass from 1951 to 1969 derived from a regression estimate of the relationship between the Murphy biomass estimate and the sardine larval index (Sections 2.4., 2.5.) determined during the reference years

o - anchovy biomass from 1940 to 1959 derived from the ratio of anchovy larvae to sardine larvae and the Murphy sardine biomass estimate

Δ - anchovy biomass from 1951 to 1969 derived from a regression estimate of the relationship between the anchovy tonnage calculated from the anchovy: sardine larvae ratio, and the anchovy larval index.

□ - Murphy estimate of anchovy spawning biomass by 3-year averages (Murphy, 1966)

Sonic survey is acquiring more and more importance owing to the recent development in acoustic instruments. Although the results obtained to date are not as numerous as those provided by the catch and effort data analysis and tagging experiments, the importance of sonic surveys in the future is expected to be extremely high. This method is used for locating fish schools and even individual fish. However, the interpretation of sonic records requires sampling by fishing operations or other means, such as photography or submersible television to identify the kind of fish. It is difficult to intercept all parts of the stock if fish are moving rapidly either horizontally or vertically unless a number of vessels are used simultaneously for a sonic survey. Methodological limitations of sonic surveys have been considered by Forbes and Nakken (1972).

There are several marked advantages in using fish eggs and larvae to monitor adult populations and estimate biomass. The early life history stages of fishes are restricted, by depth, usually to the upper mixed layers. The passive eggs and feebly swimming larvae are quite vulnerable to capture. Many marine fishes have pelagic eggs and most have pelagic larvae. Thus, it is easy to quantitatively sample several species over broad areas with a simple plankton net. This type of gear can be handled by a wide variety of vessels without major installations of equipment. The increase in cost for adding species to be studied for biomass estimates is much less when eggs and larvae are used than when adults are used. The index of larval abundance obtained from ichthyoplankton surveys has been shown to provide a reliable estimate of biomass, e.g., for sardine and anchovy (Fig. 1.1) and Pacific mackerel (Fig. 1.2).

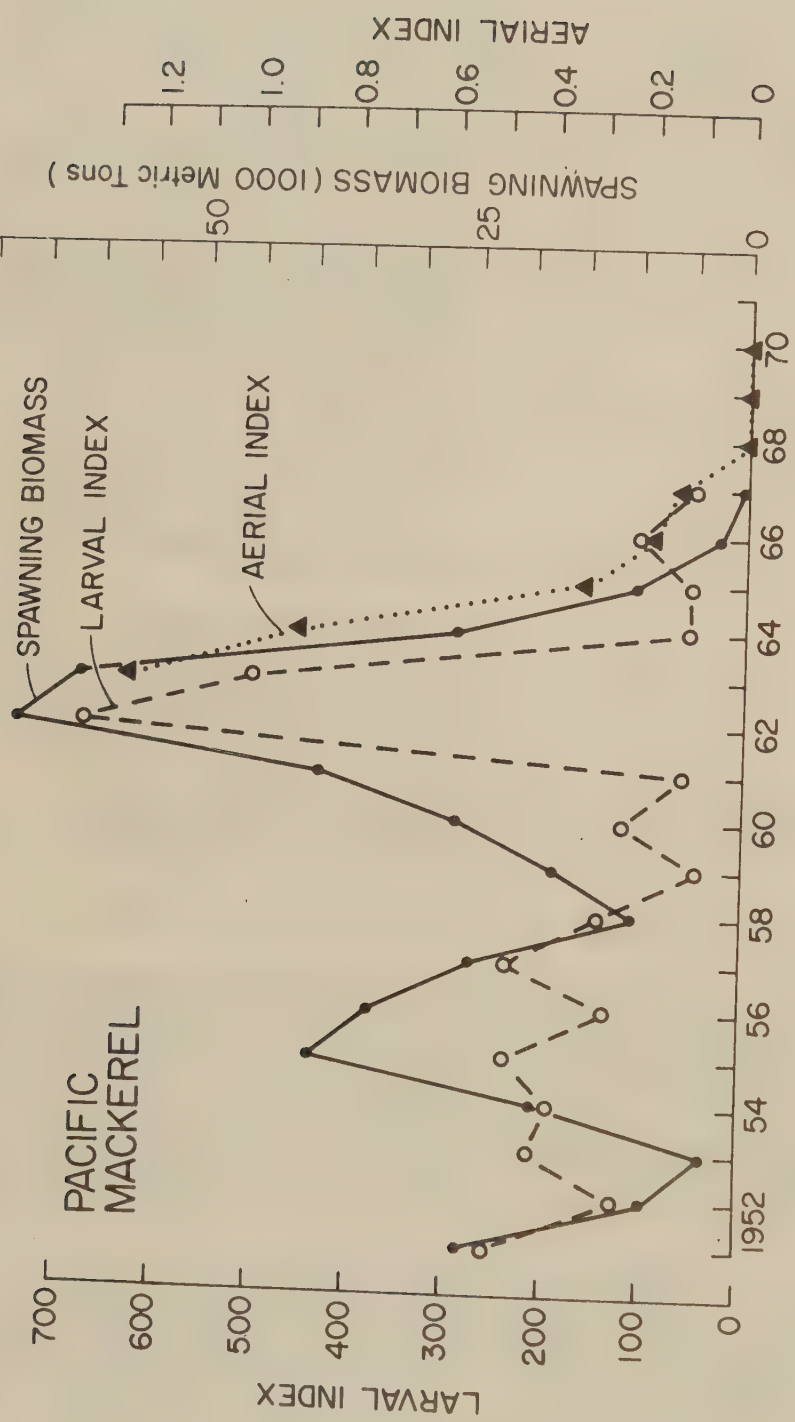


Figure 1.2. Three independent estimates of the stock of Pacific mackerel off Southern California and Baja California, Mexico.

——— estimate of spawning biomass derived by Parrish (1974) from age-specific catch and effort data using methods of Murphy (1966)

----- larval index based on CalCOFI ichthyoplankton surveys (Section 2.4.)

..... aerial index based on the number of schools logged from the air (Squire, 1972).

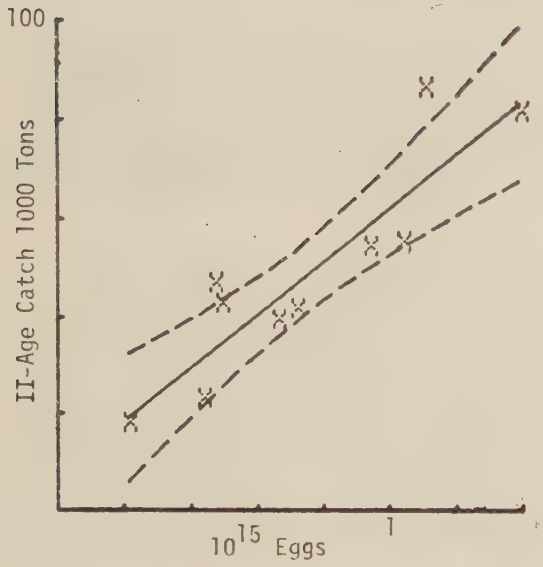


Figure 1.3. Correlation between the abundance of eggs and the adult catch of anchovy in the following fishing season in the Pacific waters along Honshu. (r_s=.91, r_d=.88). (Data from Hayashi, 1961).

There are additional benefits to be obtained from ichthyoplankton surveys primarily designed to estimate spawning biomass. The samples contain not only fish eggs and larvae, but also part of their potential zooplanktonic food and predators. Modern oceanographic techniques allow simultaneous measurements of the physical and chemical environment of planktonic communities. Trends of water motion can be estimated. The survey can detect spatial and temporal isolation efficiently over wide oceanic areas and thereby help define unit stocks of fish upon which fishery management normally depends (Gulland, 1969). Spawning distributions delimited by surveys tell when and where the fish will be concentrated for efficient capture. Results from the survey can be used for monitoring changes in the species composition and diversity of the communities in which the fish stocks reside. Data obtained can be used for forecasting the stock size into the next fishing season (Fig. 1.3.) and for forecasting the year class strength of a species. Ichthyoplankton surveys can also contribute information on new stocks of fish with commercial potential (Ahlstrom, 1968, Gulland, 1970).

Problems associated with the use of ichthyoplankton surveys to estimate spawning biomass are of a taxonomic, technical, and statistical nature. Problems involved with identification stem from the fact that early life history stages have been described in the literature for relatively few of the species. Also, there are a limited number of specialists who are broadly knowledgeable in the identification of fish eggs and larvae. These problems are surmountable (Sections 2.2., 4.). Technical problems involved with the survey require an adequate staff be assembled to handle the collection and processing of samples which may be extensive. Also, high standards must be maintained during the sampling process for quantification and comparison of data (Sections 2.1.2., 3.2.). The statistical problems are inherent in the oceanic sampling process and require the ability to interpret difficult distributions of sample data (Section 3.1.).

1.2. Scope

This manual describes recommended standard procedures for conducting quantitative ichthyoplankton surveys for spawning biomass estimates. This description (Section 2.) covers survey planning and field operations, laboratory procedures, data summarization, making census estimates, and estimating the spawning biomass. Theoretical considerations of the problems and biases involved with the recommended procedures are discussed in Section 3.

A large selected bibliography on ichthyoplankton surveys (FAO Fisheries Circular 706) is being prepared to complement this manual. In addition to papers on survey methods and results, the bibliography includes references on species identification, which is outside the scope of this manual, and tables of cross references of the papers on survey results and species identification by geographic area and taxonomic group. In cases where there are no published works and fish must be reared for identification purposes, the bibliography of May (1971) should be consulted for references on appropriate culture techniques.

2. Recommended Procedures for Conducting Ichthyoplankton Surveys for Spawning Biomass Estimates

The chief barriers to the successful execution of surveys and delivery of survey results are 1) underestimation of the necessary technical effort, and 2) imprecise definition of survey objectives. Most new ichthyoplankton surveys will encounter a mixture of new problems, specific to the survey area, and problems which have been well-defined and solved in older surveys. This section of the manual will be directed toward the standard methods recommended on the basis of survey experience so that the newer surveys may best encounter and solve the problems specific to the new survey environment.

The planning stage is likely to be the most important part of the survey for this is where the objectives of the survey are compared with monetary and personnel resources. The commitment of money and time to the surveys is an irreversible step. To minimize risk, it is necessary to set phased re-evaluations of objectives and survey performance. This process should be incorporated into the planning so that the tactics of the survey may evolve toward the most effective delivery of survey results.

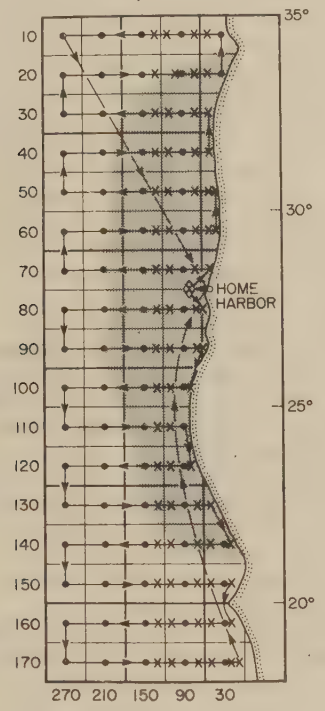


Figure 2.1. An example of a centric systematic area sample grid (Milne, 1959) superimposed on a hypothetical coastal spawning area. The solid dots represent complete biological oceanographic sampling stations and the "X's" represent supplementary stations for detailing coastal gradients. The lines connecting the stations represent a possible method of occupying each station with two ships. The diamond-enclosed "X" represents a test station occupied by both ships on each cruise to check the completeness and operability of the ship's survey equipment and to compare the performance of each ship's measuring equipment.

2.1. Field Operations

In this example, it is considered that the field operations will be conducted from ships 15 to 100 meters in length which are equipped to make plankton tows and hydrographic observations.

2.1.1. Cruise Plan and Personnel

Normally the cruise plan (Section 3.3.) is prepared by the chief scientist and the chief marine technician. After the cruise track is prepared, it is discussed with the ship's captain. Figure 2.1 shows a two-ship cruise plan to measure the extent and intensity of spawning in the outlined area.

Log sheets ("Captain's sheets") which list the desired stations and their positions, the order in which the stations are to be occupied, the captain and the navigator for the cruise ship, the month and year of the cruise, and the name of the ship (Fig. 2.2) are filled out by the chief scientist and the chief marine technician before each cruise. During the cruise, information is added to these sheets including station position actually occupied, the day of the month, the time of arrival to and departure from the station, and the method for locating the station position. Omitted stations are noted and the order in which the stations were actually occupied is corrected accordingly.

LIST OF DESIRED AND OCCUPIED STATION POSITIONS

UNIVERSITY OF CALIFORNIA
SCRIPPS INSTITUTION OF OCEANOGRAPHY

CAPTAIN	NAVIGATOR	MONTH(S)	YEAR	VESSEL	SHEET NO	OF SHEETS	CRUISE
L. E. DAVIS	K. PULSIFER	FEBRUARY	1972	AGASSIZ	1	5	7202_G

STATION	ORDER IN WHICH STATIONS WERE OCCUPIED	DESIRED POSITION		ACCEPTED POSITION OF OCCUPIED STATION		TIME (PST)			✓	REMARKS
		N LATITUDE	W LONGITUDE	N LATITUDE	W LONGITUDE	DAY OF THE MONTH	ARRIVAL	DEPARTURE		
		° '	° '	° '	° '					
90.28	1	33 28 5	117 46 7	33 28.3	117 47.0	2-8	1130	1537		Radar
90.32	2	33 20 5	118 03	33 20.7	118 02.6	2-8	1656	1812		Radar
90.37	3	33 11	118 22 5	33 11.1	118 22.5	2-8	1956	0020		Radar
90.45	4	32 54 5	118 55 5	32 54.8	118 55.8	2-9	0307	0552		Radar
90.53	5	32 39	119 28 5	32 38.6	119 28.2	2-9	0835	1040		Loran/Radar
90.60	6	32 25	119 57 5	32 24.6	119 57.4	2-9	1305	1636		Loran/Celestial
90.70	7	32 04 5	120 38 5	32 06.0	120 36.3	2-9	2002	2215		Loran
90.80	8	31 44 5	121 19 5	31 43.5	121 19.3	2-10	0145	0450		Loran
90.90	9	31 24	122 01	31 21.0	121 58 0	2-10	0800	0940		Loran
90.100		31 05	122 39							
90.120		30 25	124 00							
83.90	10	32 34 5	122 50	32 36.5	122 54.0	2-10	1829	1956		Loran
83.80	13	32 54	122 08	32 53.0	122 07.8	2-11	1100	1225		Loran
83.70	14	33 14 5	121 26	33 14.4	121 25.8	2-11	1600	1720		Loran
83.60	17	33 34	120 45	33 33.8	120 45.4	2-12	0826	1108		Loran
83.55	18	33 44	120 24 5	33 44.0	120 24.8	2-12	1300	1407		Loran

S.I.O. M.L.P. AUX. FORM 4

LA JOLLA AND POINT LOMA, 1952

Figure 2.2. List of desired and occupied station positions, the "Captain's sheets". (Courtesy University of California, Scripps Institution of Oceanography.)

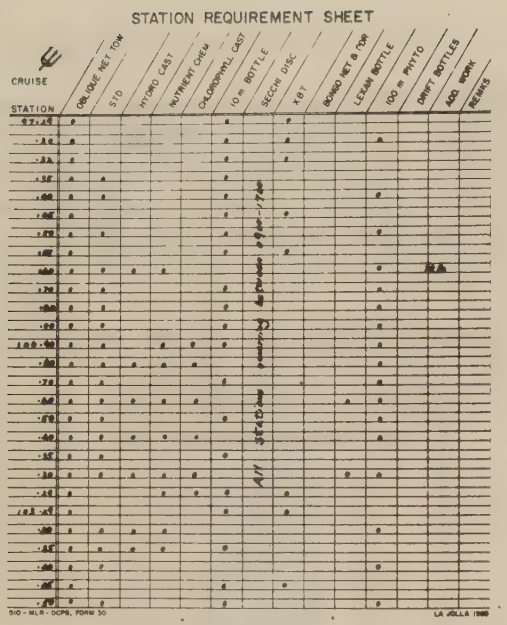


Figure 2.3. Station requirement sheet. (Courtesy University of California, Scripps Institution of Oceanography.)

The chief scientist and the chief marine technician also fill out a station requirement sheet, (Fig. 2.3.) which lists the stations the same as the "Captain's sheets" but includes additional information of the number and type of observations to be carried out on each station. Copies on this sheet are provided to each cruise leader (one of whom may be the chief marine technician). Following the description of the cruise on the station requirement sheet, the chief marine technician and the cruise leader select the marine technicians and watch leaders required for each cruise. Following the selection of the scientific staff for the cruise, a cruise announcement is issued which provides the beginning date and the proposed final date of the cruise, the objectives and procedures for the cruise and the personnel who are to conduct the cruise.

It must be stressed that having an experienced, responsible marine technician (who may also serve as cruise leader) is essential to the success of the survey. Duties assigned to the chief marine technician go far beyond those involved with setting up the cruise plan. He is responsible for seeing that the station procedures (Sections 2.1.2., 2.1.3., and 2.1.4) are carried out correctly for each station that is occupied.

2.1.2. Sampling System

The recommended sampling system requires that the ship be equipped with a hydrographic winch with more than 400 meters of standard hydrographic wire (i.e., 0.48 cm in diameter), a meter block or a metering system on the winch to measure the amount of wire out and an angle indicator (inclinometer) to measure the angle at which the wire enters the water.

2.1.2.1. The Sampler

The Bongo net (Figs. 2.4 and 2.5) towed at slow speeds, is recommended as the best type of simple gear fished from hydrographic winches for ichthyoplankton surveys. It provides a minimum of variation in the biases caused by uneven filtration per unit depth, avoidance of the net, and escapement or extrusion of organisms through the meshes. This recommended "slow" Bongo equipment is derived from bridle-free plankton nets (McGowan and Brown, 1966). It incorporates simplifications from the original opening-closing design suggested by Posgay, Marak, and Hennemuth (1968) as well as modifications made by Smith, Thrailkill, and Vrooman (1971)* after extensive comparison of the Bongo net and the CalCOFI standard net (Vrooman, 1972).**

The Bongo towing frame (Fig. 2.4) made of anodized aluminum, consists of two circular frames, each 0.6 m in diameter, connected by a central yoke to which the towing

---

* Cruise Report. David Starr Jordan #59. Dated April 1971. On file at Southwest Fisheries Center, La Jolla, California.

** Cruise Report. David Starr Jordan #70. Dated June 1972. On file at Southwest Fisheries Center, La Jolla, California.

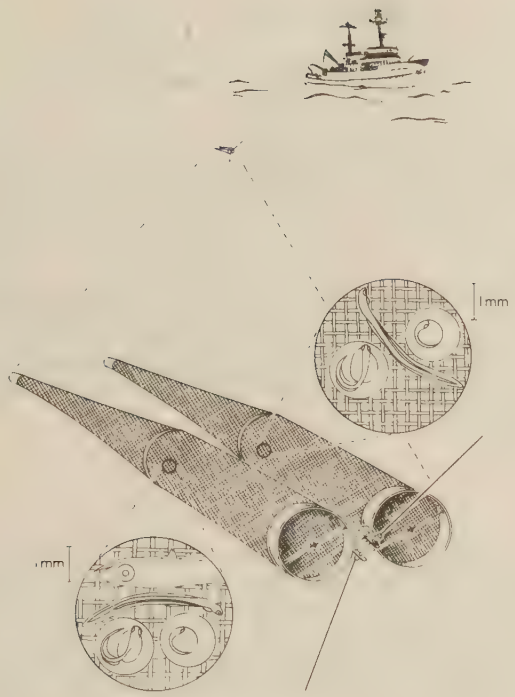


Figure 2.4. The Bongo net recommended for ichthyoplankton sampling.

A flowmeter is mounted in the mouth of each net (Fig. 2.5) to provide data on the volume of water filtered during each tow. This information is essential for quantification of the data.

2.1.2.2. The Towing Procedure and Data Records

The standard towing procedure is modified from Kramer et al. (1972). An example of an on-deck arrangement for making plankton tows is provided in Fig. 2.7.

The data sheet - Before the station is occupied, the following numbered items are recorded on the plankton-tow data sheet, a sample of which is in Fig. 2.8. (The data sheets are made of a water resistant linen which will not deteriorate when handled or when wet.)

Item 1 Cruise

Item 3 Date

Item 4 Order occupied

Item 5 Station

Item 11 Net number (= mesh size) for regular and fine mesh.

Item 12 Meter number (= flowmeter number) for regular and fine mesh.

wire is attached. Thus there are no bridles in front of the mouth of the net. A 22 kilogram dead weight depressor is suspended beneath the frame (Fig. 2.5) for making standard oblique tows.

The towing frame is fitted with two cylindrical-conical nets (Figs. 2.4, and 2.5) made of Nitex or equivalent monofilament netting preferably of a dark color. One net, which is the principal ichthyoplankton sample net, has a 0.505 mm mesh. The other net, which may be used for plankton biomass studies or fish egg and larva escapement and extrusion studies (Lenarz, 1972), should have a 0.333 mm mesh. The use of "soft" cod ends (Fig. 2.6) is recommended primarily as a matter of handling ease. Sample spillage back into the net is less likely to occur than with a "hard" cod end. The cod ends are made of the same type netting and mesh size as the main part of the net. The nets and the cod ends are color-coded for easy recognition and match up of mesh size (e.g., red for the .505 mm mesh and blue for the .333 mm mesh).

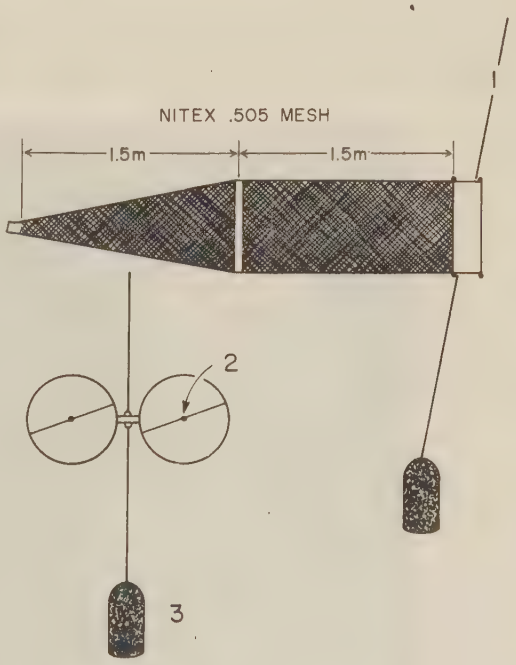


Figure 2.5. Diagrammatic view of the recommended Bongo net: 1) 0.48 cm (3/16 in.) cable from meter block on research vessel, 2) flowmeter suspended within the mouth of each net, 3) 22 kg. (50 lb.) hydrographic weight.

Item 14. Initial flowmeter reading (carryover from last tow) for regular and fine mesh.

Additional data to be recorded during the tow are mentioned as the towing procedure is described.

The tow - After the gear is assembled and the data sheet for the plankton tow is prepared (as outlined above), the tow is ready to begin. The net tow is made off either side of the ship as follows: (Tows off the stern are not recommended because of turbulence from the ship's propeller.)

1. The ship is stopped on station. Bottom depth on station is requested from the bridge and recorded in the lower left-hand section of the data sheet. Note: The bottom depth will determine the depth to which the nets will be lowered and thus the length of wire to be let out. To lower the net to 210m (the standard procedure for this type of survey, depth permitting) with a wire angle of 45° requires that 300m of wire be let out (wire angle is defined as deviation from the vertical) :

Length of wire out X cosine 45° = net depth

300m X 0.707 = 210m

If station depth is less than 238m, reference to the "depth of tow" graph (Fig. 2.9) will quickly give the proper amount of wire to be let out so that the net will not hit the bottom. For shallow tows, the wire is let out and retrieved at the same rate as for routine standard tows to avoid relative over-sampling of the surface layers. The standard tow filters 1 to 2 cubic meters of water for each meter depth. Alteration in the rate at which the wire is let out or retrieved would materially affect the basic statistical distribution of sample size.

2. The flowmeter is read and checked against the recorded initial meter reading (Item 14 on the tow data sheet). If there had been a previous tow, this should have been the final meter reading (Item 13 on the previous tow data sheet). If the reading changed between tows, the last recording is crossed out, the new reading is

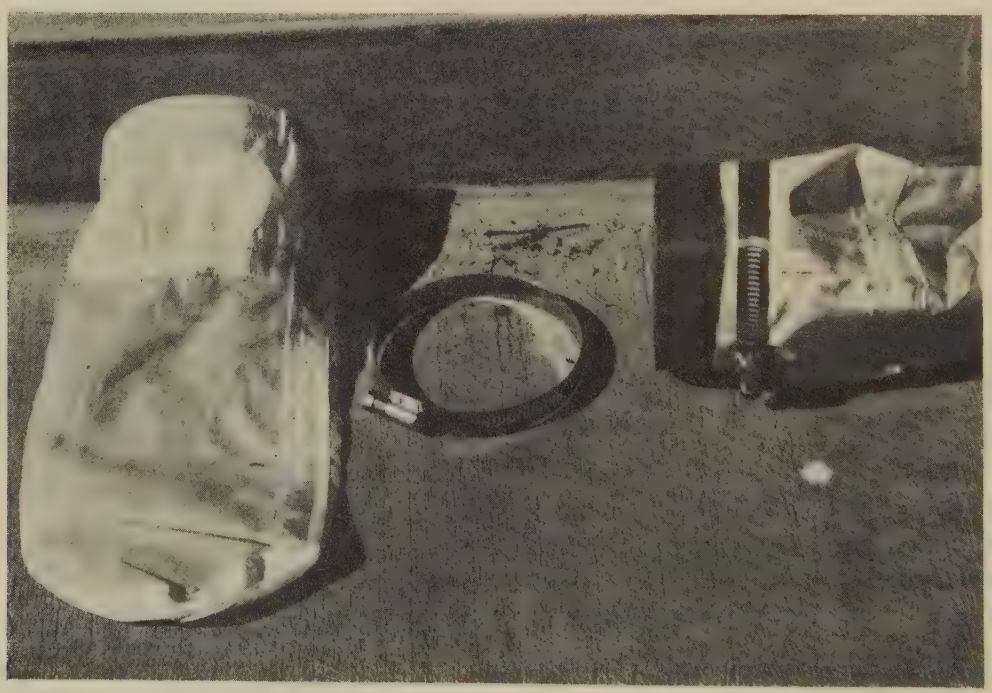


Figure 2.6. "Soft" cod end made of Nytex. (From Kramer et al., 1972)

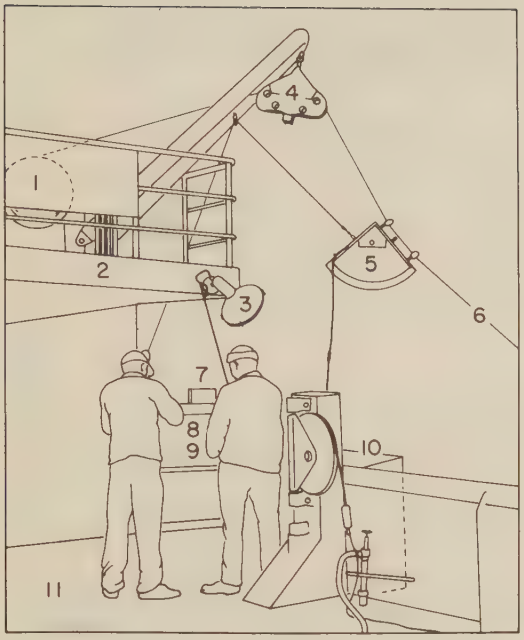


Figure 2.7. The on-deck layout of the ichthyoplankton towing apparatus on the U.S. National Marine Fisheries Service research vessel David Starr Jordan. 1) Winch drum which holds the hydrographic wire. 2) Boat deck. 3) Flood lamp for illuminating immediate area of inclinometer and overside platform. 4) Meter block which is connected to readouts in ship's bridge, dry laboratory and, and winch console and gives an instantaneous reading of meters of wire out. 5) Inclinometer which indicates angle of stray of towing wire. 6) Towing wire which is 0.48cm (3/16 in.) in diameter. 7) Intercommunications device which allows communication between bridge and winch operator during tow. 8) Meter block readout on winch console. 9) Winch and boom controls at winch console. 10) Overside work platform for hooking up inclinometer and, launching and retrieving Bongo net. (Adapted and modified from Kramer et al., 1972)

entered, and an explanation is given in the lower right-hand part of the data sheet under "Remarks".

3. The 22 kg weight is lowered below the surface of the water. If the ship is still slightly underway, the wire (#6 in Fig. 2.7) is pulled to the side of the work-platform (#10 in Fig. 2.7) and fastened close with a snap hook attached to the outside rail.

4 The inclinometer is fastened to the wire above the Bongo net (Fig. 2.7). Enough slack is left on the line to the inclinometer so that when the proper angle is achieved during the tow, it will not ride up on the cable to hit the block. (If the survey is for net tows only, the inclinometer may be left on the tow wire.)

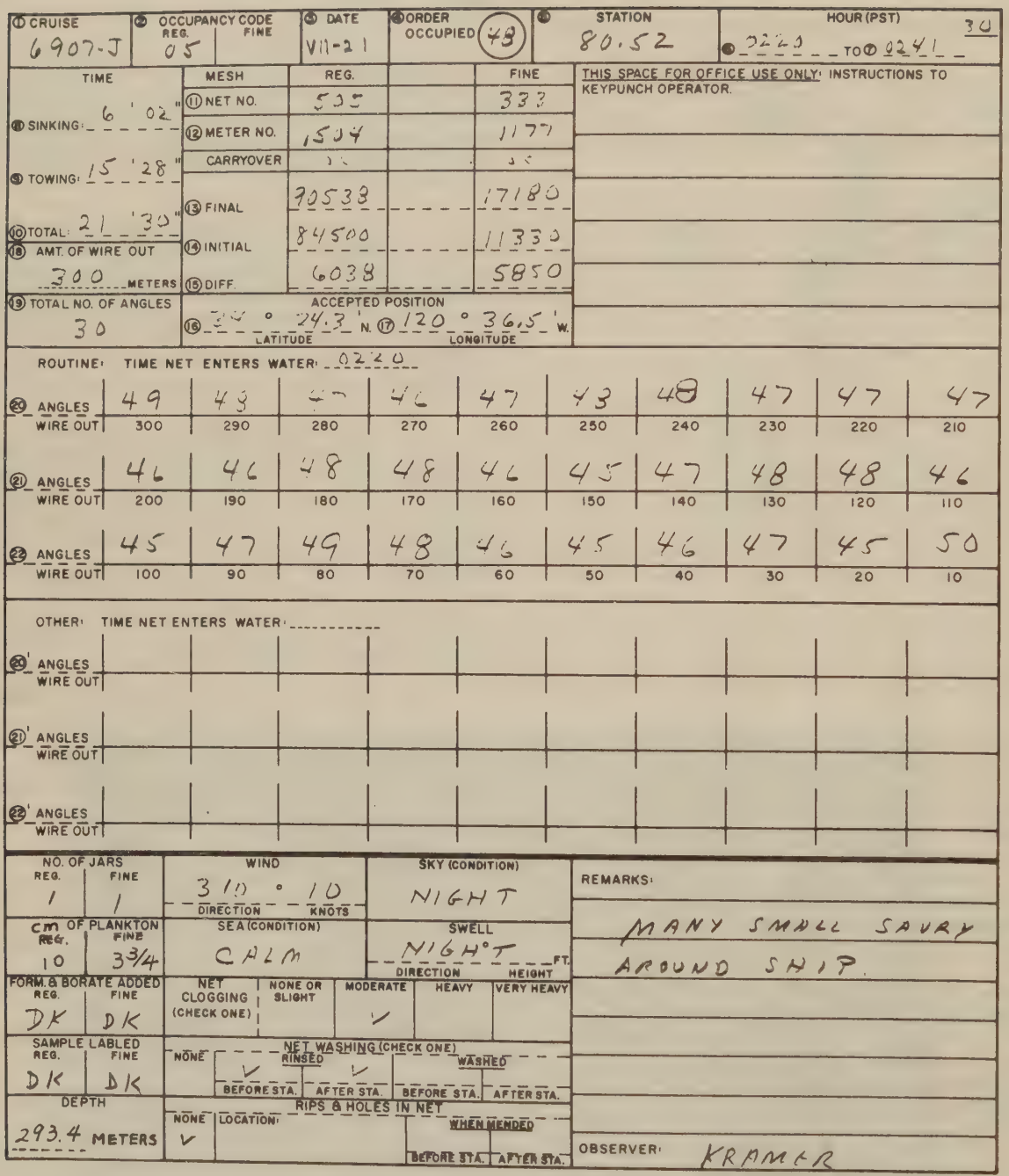
5. The winch meter is zeroed. The ship is set underway, wind off the bow on the side on which the tow is taken, and the signal to start the tow is given from the bridge. The blocks or pins, which keep the blades of the flowmeters from revolving between tows are removed, and the Bongo net is lowered into the water. (Some flowmeters have "automatic" blocks that release the impeller blades when water flows through them.)

a. The nets are allowed to stream out briefly before lowering. As soon as it is obvious that they are not tangled, the wire is immediately let out the predetermined amount at a constant rate of 50 m/min.

b. The stopwatch is started as soon as the flowmeters are seen to sink below the surface of the water. The stopwatch is used to record sinking time (Item 8) and towing time (Item 9) in seconds. The duration of the tow in seconds is used for calculation of mean velocity of towing.

c. The time the net enters the water is recorded in nautical time (2400 hr) to the nearest 5 min. (Item 6, and Item 20 or 20' depending on whether the tow is "Routine" or "Other"). The time of day is used for analysis and correction of day-night differences in the catching power of the net.

d. When the desired amount of wire has been let out, the stopwatch is stopped, the sinking time is recorded in seconds (Item 8), the



① CRUISE: 6907-J	② OCCUPANCY CODE REG.: 05 FINE:	③ DATE: VII-21	④ ORDER OCCUPIED: 48	⑤ STATION: 80.52	HOUR (PST): ⑥ 2220 TO ⑦ 0241 — 30

TIME	MESH	REG.		FINE
⑧ SINKING: 6 '02"	⑪ NET NO.	535		333
	⑫ METER NO.	1504		1177
⑨ TOWING: 15 '28"	CARRYOVER	〃		〃
	⑬ FINAL	70538		17180
⑩ TOTAL: 21 '30"	⑭ INITIAL	84500		11330
⑱ AMT. OF WIRE OUT: 300 METERS	⑮ DIFF.	6038		5850

⑲ TOTAL NO. OF ANGLES: 30

ACCEPTED POSITION: ⑯ 34 ° 24.3 ' N. LATITUDE ⑰ 120 ° 36.5 ' W. LONGITUDE

THIS SPACE FOR OFFICE USE ONLY: INSTRUCTIONS TO KEYPUNCH OPERATOR.

ROUTINE: TIME NET ENTERS WATER: 0220

⑳ ANGLES	49	43	47	46	47	43	48	47	47	47
WIRE OUT	300	290	280	270	260	250	240	230	220	210
㉑ ANGLES	46	46	48	48	46	45	47	48	48	46
WIRE OUT	200	190	180	170	160	150	140	130	120	110
㉒ ANGLES	45	47	49	48	46	45	46	47	45	50
WIRE OUT	100	90	80	70	60	50	40	30	20	10

OTHER: TIME NET ENTERS WATER: ----------

⑳' ANGLES										
WIRE OUT										
㉑' ANGLES										
WIRE OUT										
㉒' ANGLES										
WIRE OUT										

NO. OF JARS REG.: 1 FINE: 1	WIND: 310 ° 10 (DIRECTION / KNOTS)	SKY (CONDITION): NIGHT	REMARKS: MANY SMALL SAURY AROUND SHIP.
cm OF PLANKTON REG.: 10 FINE: 3 3/4	SEA (CONDITION): CALM	SWELL: NIGHT ° FT. (DIRECTION / HEIGHT)	
FORM. & BORATE ADDED REG.: DK FINE: DK	NET CLOGGING (CHECK ONE): NONE OR SLIGHT / MODERATE ✓ / HEAVY / VERY HEAVY		
SAMPLE LABLED REG.: DK FINE: DK	NET WASHING (CHECK ONE): NONE / RINSED BEFORE STA. ✓ / RINSED AFTER STA. ✓ / WASHED BEFORE STA. / WASHED AFTER STA.		
DEPTH: 293.4 METERS	RIPS & HOLES IN NET: NONE ✓ / LOCATION: / WHEN MENDED BEFORE STA. / WHEN MENDED AFTER STA.		OBSERVER: KRAMER

Figure 2.8. Plankton tow data sheet. (From Kramer et al., 1972). A sample copy of a sheet made out for station 80.52 on cruise 6907-J.

stopwatch is zeroed and restarted immediately. (Since the net is "fishing" on the way down, sinking time is as important as that of retrieval.)

e. When the stopwatch is restarted, the nets are left at the desired depth for 30 sec. (hypothesizing that "falling" nets will straighten out at depth in the 30-sec. interval).

f. At the end of 30 sec. the stopwatch is not stopped, the wire angle is recorded for that depth, and retrieval is begun at the rate of 10 m per 30 sec. for all tows. The wire angle is recorded at every 10 m (Item 20 to 22 for routine tows with 300 m of wire out or Items 20' to 22' for other tows). Note: Ship speed, during sinking, during times at depth, and during retrieval, is maintained to keep the wire angle at 45°. In dead calm, it may be necessary to run the ship in circles to maintain the wire angle. It is essential to maintain the 45° (±3°) wire angle to assure that the proper amount of water is being filtered from each depth (1 to 2 cubic meters per each meter depth) for purposes of data quantification.

g. The nets are brought directly out of the water at a steady rate. Note: It is important not to allow the nets to fish too long at the surface because of the bias that results from oversampling surface waters (Section 3.2.1.). When the flowmeters break the water surface, the stopwatch is stopped and its reading in seconds is recorded as the towing time (Item 9). Note: If the flowmeters are the kind that do not have "automatic" blocks on the impeller blades, blocks or pins should be placed in them to stop the impeller blades from rotating as soon as possible after the flowmeters break the water surface.

6. The nets are washed down from the outside to get all the plankton into the cod ends, keeping the net rings at rail height with the cod ends dangling. A salt water hose is used for the washing. Water pressure should be enough to loosen the plankton adhering to the net mesh but not so much as to damage the plankton organisms. This operation usually takes only a few minutes per net.

7. After all the plankton has been washed into the cod ends, the nets are brought aboard. The color-coded cod ends (e.g., red for .505 mm mesh and blue for 0.333 mm mesh) are removed, keeping the plankton from spilling back into the nets. The plankton samples are taken to the ship's wet laboratory and preserved immediately (Section 2.1.3.1.).

8. After the samples have been preserved, labelled, and stored, the cod ends are washed (they may be left everted) and replaced on the nets with matching color codes in preaparation for the next tow.

9. Before leaving the station, the flowmeters are read and recorded as the final readings (Item 13). The initial readings (Item 14), which were made before the tow began, are subtracted from the final readings (Item 13) to get the differences (Item 15) which are the number of revolutions for the tow for each net. Note: It is important that the proper amount of water has been filtered for quality of tow and data quantification. For each flowmeter, the proposed central number of revolutions and acceptable limits to this number of revolutions should be calculated before the cruise to serve as a guide for the observer to tell if the tow has been correctly conducted. If meter readings are not normal, the net tow may have to be repeated. A very high reading may have been caused by too great a ship's speed - check for many high wire angles. A low reading may have been due to too slow a ship's speed - check for many low wire angles. Another reason for low meter readings may be clogging of the nets. This may be cumulative if a net is not rinsed properly or it may occur at a single station. If a meter shows a trend toward lower and lower readings, and it is not malfunctioning, the net should be washed (Section 2.1.2.2., #15). The net tow need not be repeated if it is obvious that heavy clogging is the reason for low readings (it will only clog again) or if the ship's speed has caused low or high angles. If wire angles are normal, the net is clean, and the towing time (Section 2.1.2.2. #11) is routine but the meter reading is low, the cause could be that a bit of detritus, a fish, or even a large jelly or salp had become entangled in the meter blades for a portion of the tow. Under these conditions, the tow should be repeated. If the

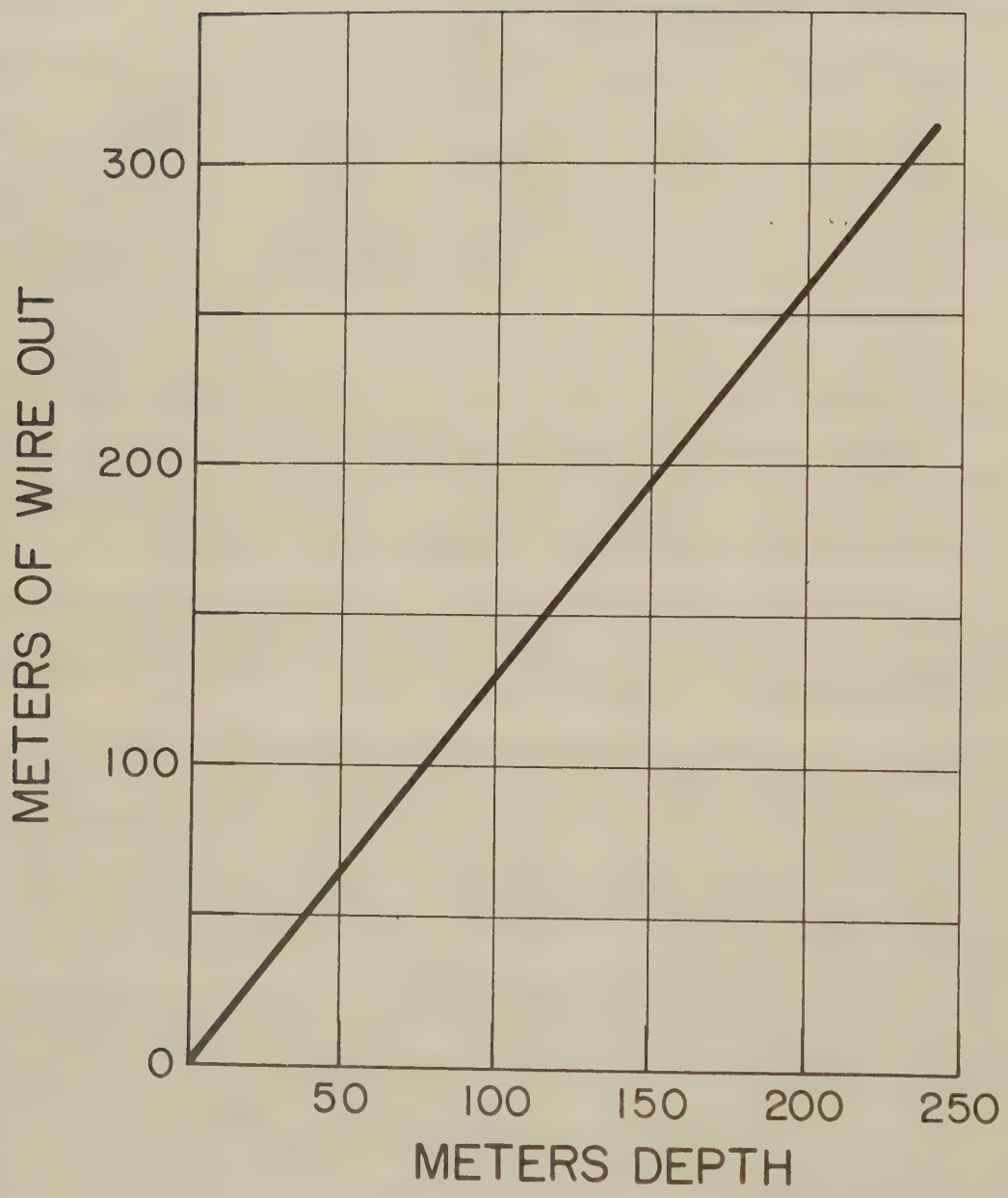


Figure 2.9. Depth-of-tow graph. (Modified from Kramer et al., 1972).

reading is again very low and it is obvious that the flowmeter is not functioning properly, replace the meter and repeat the tow. Do not oil or grease any meter or make any repairs that might alter the rotation of the blades. Repairs of this type would seriously affect the calibration of the meter. If clogging is apparent, the amount is recorded in the appropriate box on the plankton tow data sheet.

10. Wind, sky, sea condition and sea swell should be requested from a crew member by the observer who is recording the angles while the tow is being made. The information is recorded in the appropriate boxes in the lower section of the plankton tow data sheet.

11. Total towing time (Item 16) is recorded as the sum of Items 8 and 9. For a 300 m tow, total time should be about 21'30" (6' sinking time + 15'30" towing time). Note: If total time is off by 15 to 20 sec., it must be explained in the "Remarks" section. The most usual variation will be in the sinking time, caused by a slightly faster or slower rate in paying out the wire than the recommended 50 m/min. In certain conditions, such as poor control of the ship, countercurrents below the sea surface adversely controlling the net as it falls, etc., the winch operator may have to depart from the sinking-time procedures to slow the falling net in order to keep it from becoming tangled. Such departures from normal procedures must be recorded in the "Remarks" section.

12. Total towing time is added, in minutes and seconds to Item 6 to record the hour, minutes and seconds in Item 7. This is actually the time the net comes out of the water.

13. The actual station position (accepted position) is recorded in Items 16 and 17. This may be done when the station is occupied, but usually is done at the end of the cruise when the captain has compiled a complete list of the positions of all stations (Section 2.1.1.).

14. Checks are made regarding the sample (Section 2.1.3.) and appropriately recorded on the tow data sheet in the lower left-hand section.

a. Number of jars per sample.

b. Centimeters of plankton - this gives approximate volume before water is added.

c. Formalin and sodium borate added - the person who adds the preservative and buffer must initial this box for each sample <u>after</u> the Formalin and sodium borate are added.

d. Sample labelled - the person who labels the sample must initial this box <u>after</u> the sample is labelled.

15. Checks are made on the net to see if washing or repair is necessary. The appropriate boxes are checked in the lower section of the tow data sheet. If replacement of a net is necessary, it should be recorded in the "Remarks" section.

a. If washing is needed, one of three methods may be used: (1) The net is everted, still on its ring, and brushed down with an ordinary sweeping broom and running sea water; (2) The rings are stood on edge, the net is tightened along its length by tying down the end (without cod end attached) and hosed down with a high-pressure fire hose. This is very effective provided that plankton has not dried in the meshes; (3) The net is detached from the ring and put in a washing machine using a 30-min. cycle, war water (not hot) and a non-polluting detergent.

b. Rips and holes in the net - the net should be examined after every tow to check on needs for repair or replacement. If holes or tears are small, they should be sewn before the next tow with nylon thread of a color (e.g., red) that can be easily located for sewing machine repair later on shore. If the net is torn beyond mending at sea, replace the net.

16. Recheck the tow data sheet to be sure that all items are filled in. (The occupancy code, Item 2, is filled in onshore at the end of the cruise. This is usually one of a series of numbers used by a computer programmer to describe the type of tow or the station occupied.) Note: Some deviations from standard procedure cannot be avoided. Circumstances such as strong under-sea currents, high winds, heavy seas, will cause unavoidable deviations, e.g., odd meter readings, prolonged stops at stations. Conditions of this kind should be noted in the "Remarks" section.

17. A new plankton tow data sheet is set up for the next station: Items 1, 3, 4, 5, 11, 12, and 14 are entered. Item 14 should be the final reading of the preceding tow and should be rechecked before starting next tow.

2.1.3. Handling the Sample at Sea

Fish eggs and larvae are fragile and easily damaged. Proper care is needed in all stages of preservation and handling plankton samples aboard ship.

2.1.3.1. Preserving the Sample

The plankton sample should be preserved immediately. This is especially critical in tropical waters.

The storage container in which the sample is preserved should be of sufficient size so that when filled, the preserving liquid (5% buffered Formalin is recommended) will occupy at least three times the volume of the plankton. No problems are posed in having a large ratio of preserving liquid to plankton. Glass jars holding 1000 ml are recommended. Normally they are closed by a screwed-on plastic lid with an inside coating to prevent leakage and evaporation.

The plankton collection is carefully poured from the cod end into the container in which it will be stored. The cod end is then rinsed down to gather the last of the plankton at its bottom. When fairly well drained, the cod end is everted over and into the jar and the remaining plankton is washed off carefully. It has to be ensured that no parts of the sample remain on the mesh of the cod end.

At this point, before seawater is added, the height of the plankton in the jar is measured in centimeters. This gives an approximate volume to be recorded on the plankton tow data sheet (Section 2.1.2.2., #14).

The jar containing the plankton is then filled three-fourths full with seawater before adding the preservative (full strength Formalin) and buffer (sodium borate). This is done to avoid "burning" the delicate plankton organisms. To obtain the recommended 5% solution of Formalin in a one-liter jar, 50 ml of concentrated commercial Formalin is added. To assure proper buffering, 20 ml of a saturated

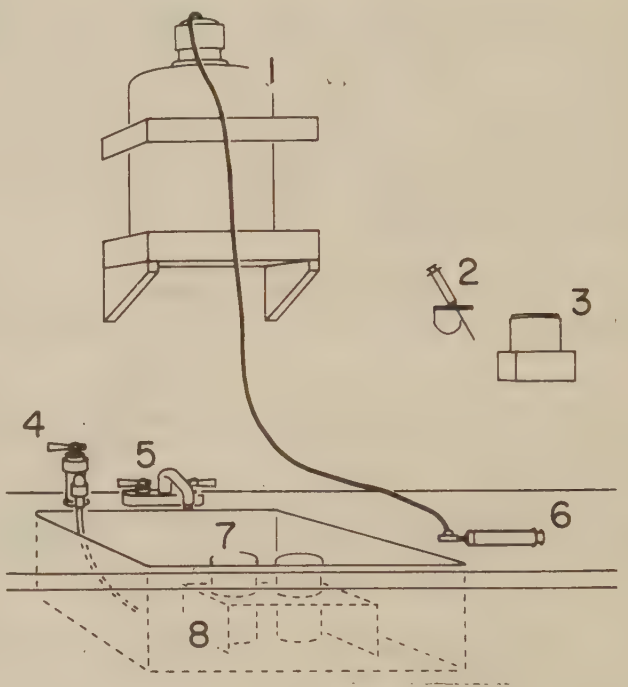


Figure 2.10. Ship's wet laboratory setup for preserving and labelling plankton samples. 1) Plastic carboy (19 liters) containing concentrated formaldehyde and having 1.5 m of surgical tubing. 2) 20 ml disposable syringe with cannula for adding buffer to plankton samples. 3) Container of sodium borate in seawater. 4) Salt water tap with 0.5 m of surgical tubing to rinse cod end, concentrate plankton, and fill sample jar after fixation and buffering. 5) Ship's hot and cold freshwater taps. 6) 50 ml disposable syringe with automatic double valve for measuring and dispensing formaldehyde. 7) 1 liter sample jars. 8) Removable wooden frame to support sample jars in rough weather. The entire assembly, including sink, can be fitted into 1 to 2 m^2 of laboratory bench space. (Adapted and modified from Kramer et al., 1972.)

solution of sodium borate in seawater is added for each liter of preserved sample. (Buffer is added to counteract the acidity of plankton in Formalin. Hexamine should definitely not be used as a buffer. A solution containing too much or too strong a buffer is harmful to fish larvae.) The sample jar is then filled almost to the top with seawater, capped and shaken lightly (including inversion) to obtain immediate, uniform preservation of the plankton organisms throughout the sample.

Data on the number of jars per sample and addition of Formalin and borate to the sample must be entered on the plankton tow data sheet (Section 2.1.2.2., #14). Note: full strength formaldehyde aboard ship is kept in 19 liter polypropylene carboys (Fig. 2.10). With the carboy moored securely above the sink, the preservative is drawn by siphon action. A further safety measure is to draw the formaldehyde via a teflon tube into a 50-ml plastic syringe through an automatic double valve. The buffer is added with a 20-ml plastic syringe fitted with cannula (a "needle" without a point).

Formalin is the most widely used preservative for plankton collections. It is by no means "ideal", but satisfactory when properly handled. Years of experience have shown that too strong or too weak a concentration of Formalin can be damaging to ichthyoplankton. The recommended concentration of 5% for preserving the sample and 3% for later storage of eggs and larvae (Section 2.2.3.) have proven to work well over a long time scale. Other recommendations have been recently proposed (Steedman, 1976; UNESCO, 1974).

SCRIPPS INSTITUTION OF OCEANOGRAPHY
ZOOPLANKTON COLLECTION

JORDAN — Ship
7203-J — Cruise
Mar-10-72 — Date
1030 PST — Loc. Time
77.55 — Station
Actual or MWO
Depth of Tow
1 m .505 — Gear and Mesh
Horiz., Oblig or Vert.
Remarks
FH — Collector

Figure 2.11. Labels for field identification of plankton samples. A. Inside paper label (Courtesy University of California, Scripps Institution of Oceanography). B. Outside label on jar lid.

The concentrated, commercial preparation of Formalin is a 40 percent solution of formaldehyde gas in water. Formalin should be stored in inert containers, glass or plastic, not in metal containers; Formalin reacts with the latter. One of the problems in storage of concentrated Formalin is its tendency to polymerize - to form para-formaldehyde, a flocculent, white solid.

It should always be kept in mind that formaldehyde is a poison that can irritate skin and cause serious cases of dermatitis. Rubber gloves should be worn when preparing formaldehyde solution, or when handling specimens preserved in Formalin. Formaldehyde fumes can also be irritating to the lungs.

2.1.3.2. Labelling

It is essential that samples be properly labelled. Information contained on the labels should be sufficient to identify the sample with certainty. Each sample jar should have two labels. One label is placed inside the jar. Because this label is often difficult to read unless removed, a second label is placed on the lid of the sample jar. Both labels are necessary because jar lids can be mixed up. Should this happen, the inside label usually assures proper identification of the sample.

The inside label (Fig. 2.11) should be made of heavy weight, chemically resistant linen paper which will not fall

apart in the Formalin solution. The label is filled out with a soft carbon pencil which will not fade in the Formalin. This label should contain the following information: name of ship, cruise number, date, time, station designation, depth of tow, gear used, mesh size, type of haul, duration of haul, collector's name, number of jars in the sample (1 of 2, 2 of 2 or 1 of 3, 2 of 3, etc.).

The outside label (Fig. 2.11) is written on the lid of each sample jar with a light-colored waterproof marker or wax pencil. It should contain the following information: cruise number, station, date, time, gear, mesh size, number of jars in the sample (1 of 3, 2 of 3, etc.). This outside label should be color-coded to match the net and cod end mesh sizes. This may be done by using an appropriately colored waterproof marker or colored tape (e.g., red for .505 mm mesh and blue for .333 mm mesh).

After the samples are labelled, the person who labels them should initial the appropriate box on the plankton tow data sheet (Section 2.1.2.2., #14).

2.1.3.3. Storage on the Vessel

Sample jars should be completely filled. This is to prevent agitation of plankton in the jars during rough weather, as will happen if they are only partially filled. Even when filled, some movement of the organisms within a jar is inevitable due to vessel motion (rolling, pitching, etc.). Hence, to minimize damage to the delicate organisms during the storage on the vessel, samples should be stored in the most stable part of the ship.

Temperature of storage may be important, particularly in tropical regions. This factor seldom has been given much attention. Collections from tropical expeditions often are of poor quality, even when care has been taken in their preservation and handling. Experiments are needed to test the effect of storage temperatures on the condition of plankton organisms, especially during long cruises. It may be found that air conditioned (temperature-controlled) storage rooms are essential for the proper care of plankton collections on tropical expeditions.

2.1.4. Supplementary Data

At the critical stages in the life history of young fish, minor changes in the environment can cause extensive mortalities and lead to corresponding fluctuations in the abundance and availability of fish stocks. It is therefore important to collect environmental information during the course of ichthyoplankton surveys.

2.1.4.1. Hydrographic Data

For the standard ichthyoplankton survey, it is important to obtain at least some physical oceanographic data at each station. This usually includes a surface temperature taken with a bucket thermometer, and more importantly a bathythermograph (BT) or expendable bathythermograph (XBT) observation to obtain a profile of temperature with depth. A sample data sheet for use with BT observations is in Fig. 2.12. (If an XBT is used, the "BT instrument number" column is left blank).

Additional observations could include samples of water for determination of salinity, oxygen, and nutrients and a Secchi disc reading at day stations.

2.1.4.2. Smaller Zooplankton and Phytoplankton

Information on the availability and utilization of the food of larvae can be obtained by simultaneously sampling for the smaller plankton constituents eaten by the larvae. For studying these organisms, it is recommended that paired Bongo nets, 20 cm in diameter at the mouth, be used. One net should be fitted with netting of 0.250 mm mesh apertures and the other with 0.150 mm mesh. These nets can be fastened to the towing cable immediately above the 60 cm Bongo assembly (Fig. 2.13). It is also highly desirable to measure photosynthetic pigments. The zooplankton in the smaller nets and the photosynthetic pigment data will provide a standard base for comparing the relative productivity of the areas investigated.

OCEANOGRAPHIC LOG SHEET-B

BATHYTHERMOGRAPH OBSERVATIONS

FOR USE WITH H.O. PUB. NO. 606-C

VESSEL: STRANGER | MONTH: Oct. | YEAR: 1957 | TIME ZONE: PST | CRUISE: 5710-S | SHEET NO. 1 OF 5

CONSEC. S. OF NUMBER	GREENWICH MEAN TIME HOUR	GREENWICH MEAN TIME DATE	LATITUDE	LONGITUDE	DEPTH (Meters)	SEA SURFACE TEMP (Bucket)	WIND DIR	WIND SPEED	AIR TEMP DRY BULB	AIR TEMP WET BULB	BAROM (cm)	WEATHER (Tab. 3)	CLOUDS TYPE (Tab. 4)	CLOUDS AMT (Tab. 5)	VIS (Tab. 6)	SEA (Tab. 7)	SWELL DIR	SWELL AMT	BT INSTR NO	OBS INIT
H-1	0734	4	30 26.0	116 11.0	468	19.3	315	4	65.0	60.9	7405	02	6	6	8	2			78848	G
H-2	1015	4	30 20.0	116 23.0	1350	19.9	350	3	65.8	61.0	7405	02	6	3	8	2			78848	G
H-3	1340	4	30 10.0	116 43.0	2790	20.2	340	5	68.3	64.2	7395	02	6	2	8	3			78938	G
H-4	1925	4	29 50.0	117 24.0	3060	20.0	300	5	66.8	68.1	7455	01	—	0	8	3			78938	DB
H-5	0040	5	29 30.0	118 02.0	3060	20.4	325	5	62.2	61.4	7465	03	0	3	8	3			78938	DB

FORM 20 OCPG

Figure 2.12. Oceanographic log sheet for bathythermograph observations. (Courtesy University of California, Scripps Institution of Oceanography).

2.1.4.3. Neuston

It is advisable to include a neuston tow for larvae and juveniles for 10 minutes during the Bongo net tow. Larvae of several species of commercially-important pelagic fishes are known to occur in concentrations in the surface layer (Klawe, 1963; Sund and Richards, 1965; Parin, 1968; Zaitsev, 1970; Richards and Simmons, 1971). Their occurrence is mainly ephemeral, varying with stage of development, light, feeding, and oceanographic conditions (see review by Hempel and Weikert, 1972). Neuston samples can be collected with relatively small effort and they can provide valuable information on a number of kinds of fishes including tunas, billfishes, scombroids, and even some flatfishes. A rough check on presence or absence of eggs, larvae, and juveniles in the surface layer can be done with any kind of simple floating net which is towed at a speed of two knots. The mouth opening should cut the water surface and extend to about 10 to 50 cm deep. The tow should be operated aside of the ship and well clear of its bow wave and wake. Mesh size should be the same as in other ichthyoplankton sampling gear, i.e., about 0.3 or 0.5 millimeters. In some surveys, including the Cooperative Investigation of the Caribbean (CICAR), simple rectangular frame nets of 2 x 1 m or 1 x 0.5 m mouth aperture have been used. More sophisticated gear is now in use at several laboratories with the objective of sampling the near-surface layer in a more quantitative manner. The major difficulty in this respect is to control the depth of insertion of the net's mouth opening into the water and hence the amount of water filtered per meter of tow. The modified David-Neuston net (Hempel and Weikert, 1972), which is now recommended for international surveys such as the Cooperative Investigation of the Eastern Central Atlantic (CINECA), Sameoto's otter surface net (Sameoto and Jaroszynski, 1969), and various gears by Zaitsev (1970) have to be mentioned in this context.

2.1.4.4. Micronekton

Data on ichthyoplankton obtained from standard Bongo net samples can be usefully supplemented by larger nets with larger mesh for studying the larger, more agile larvae and juveniles. The objectives of these larger nets are to reduce the degree of avoidance, increase the total volume of water filtered, and reduce the amount of plankton from which the larger larvae and juveniles must be sorted. One such net has been designed for the National Marine Fisheries Service (NMFS) Marine Resources Monitoring Assessment and Prediction (MARMAP) program. The towing frame is the 6-foot Isaacs-Kidd midwater trawl (IKMT) with 2.89 m^2 mouth area, uniform 2 mm knotless mesh, with five times as much

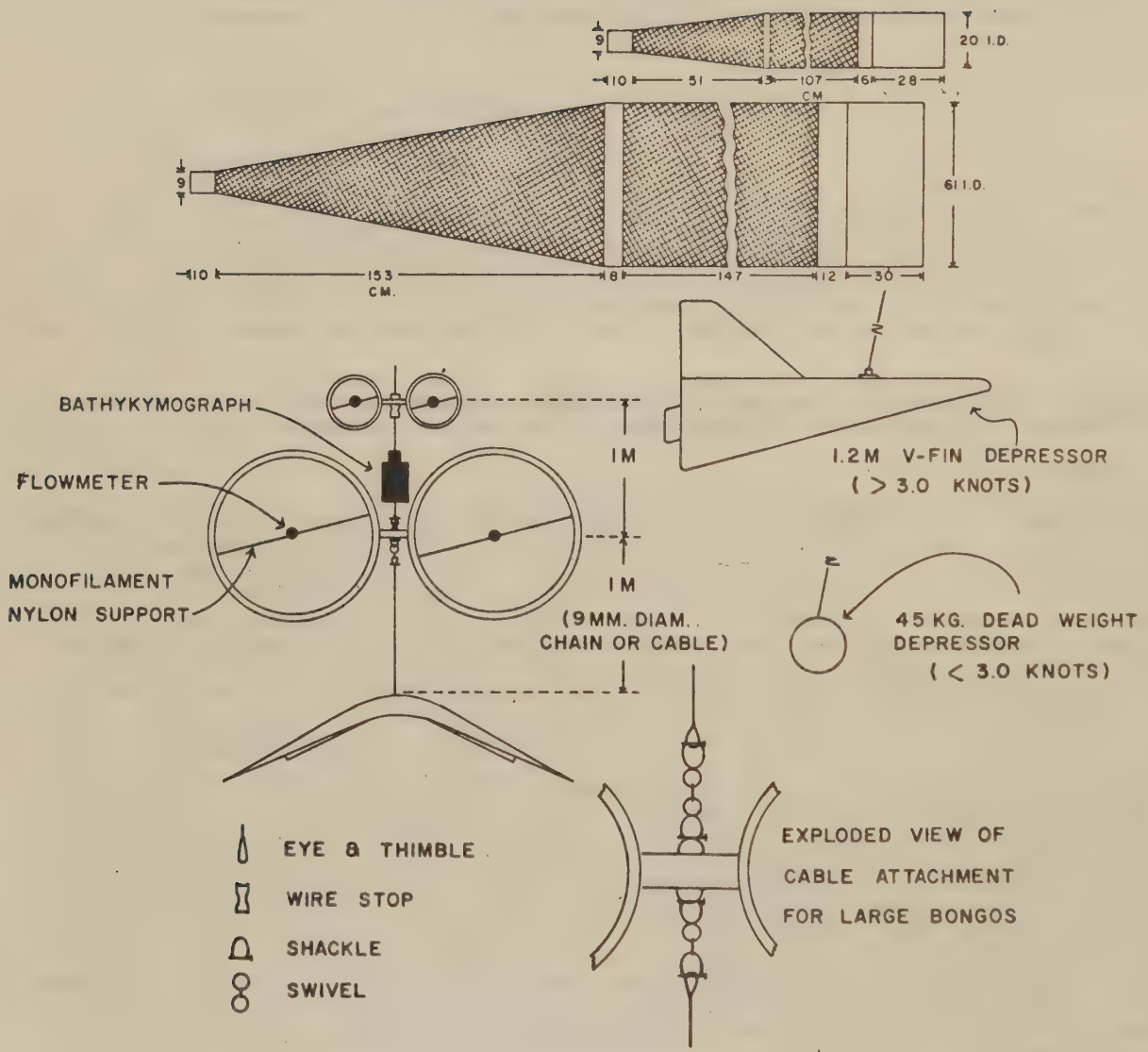


Figure 2.13. Paired Bongo net array with four mesh sizes; 0.505 mm, 0.333 mm, 0.250 mm, and 0.150 mm.

½ m 333

Plankton Volumes Cruise: 6910-J 5 of 7

Station	Ord. Ocpd.	No. Jars	Person Meas.	Total Volume	T.V.-L. Org.	% Fract.	Fractioned Samples Vol. L	Vol. R	Vol. Rem.	Person Sorting	Date Sorted	Remarks
87.35	72	1	JDM	26	26					NHG	9-6-72	
·40	71	1	JDM	44	44					NHG	9-7-72	
·45	70	1	JDM	21	21					NHG	9-7-72	
·50	69	1	JDM	13	13					NHG	9-7-72	
·55	68	1	JDM	9	9					NHG	9-7-72	
·60	67	1	JDM	106	12					NHG	9-7-72	94 cc Salps
·70	66	1	JDM	185	185					NHG	9-8-72	
·80	65	1	JDM	34	34					NHG	9-8-72	
·90	64	1	JDM	119	110					NHG	9-8-72	9 cc. Large org.
90·28	74	1	JDM	14	14					PSL	9-6-72	
·32	75	1	JDM	13	13					PSL	9-6-72	
·37	76	1	JDM	25	25					PSL	9-6-72	
·45	77	1	JDM	34	34					PSL	9-6-72	
·53	78	1	JDM	17	17					PSL	9-6-72	
·60	79	1	JDM	27	27					PSL	9-6-72	

Figure 2.14. Plankton volume data sheet. (Modified from Kramer et al., 1972)

filtering area as mouth area. The present (1973) use of the net is in an oblique tow from 200 m to the surface filtering 13,000 m^3 of water at 4 knots towing speed. The duration of the tow is 35 minutes. All samples are taken at night. The early experimental work is being conducted in conjunction with a 75 m^2 otter board trawl with 4 mm mesh to discern the upper limits of effective sampling of fish larvae and juveniles in the 6-foot IKMT. The MARMAP IKMT is not designed for opening and closing at depth. The Tucker net, with square mouth opening, can be modified into an effective opening-closing micronekton net (Davies and Barham, 1969).

2.2. Laboratory Procedures

This section describes laboratory techniques employed in processing plankton samples, collected specifically for fish eggs and larvae, to be used for spawning biomass estimates. Determining the number and kind of fish eggs and larvae present in each sample is of primary interest. A secondary interest is the other plankton constituents as they relate to the well-being of the young stages of fishes, either as prey (food) or predators. The samples may also be used for other studies, e.g., the taxonomy of the various constituents, plankton community structure, indicator organisms, zoogeography, life history studies, etc., which are beyond the scope of this manual.

2.2.1. Plankton Volume Determinations

A measurement of wet plankton volume, determined by displacement, is made for each plankton sample soon after the samples are taken ashore. This data is recorded for each cruise on a plankton-volume data sheet (Fig. 2.14). The plankton volume measurement provides a rough measure of zooplanktonic biomass (Ahlstrom et al., 1969). It also has a practical value in the subsequent handling of the sample. Large samples may have to be aliquoted for sorting (Section 2.2.2.) and the size of the aliquot will often depend on sample size.

Two volumes (Fig. 2.14) are reported for each sample. The total volume includes everything in the sample except adult fishes, juvenile fishes, larger squids, octopi, and adult pelagic crabs (such as Pleuroncodes) which are not considered planktonic.

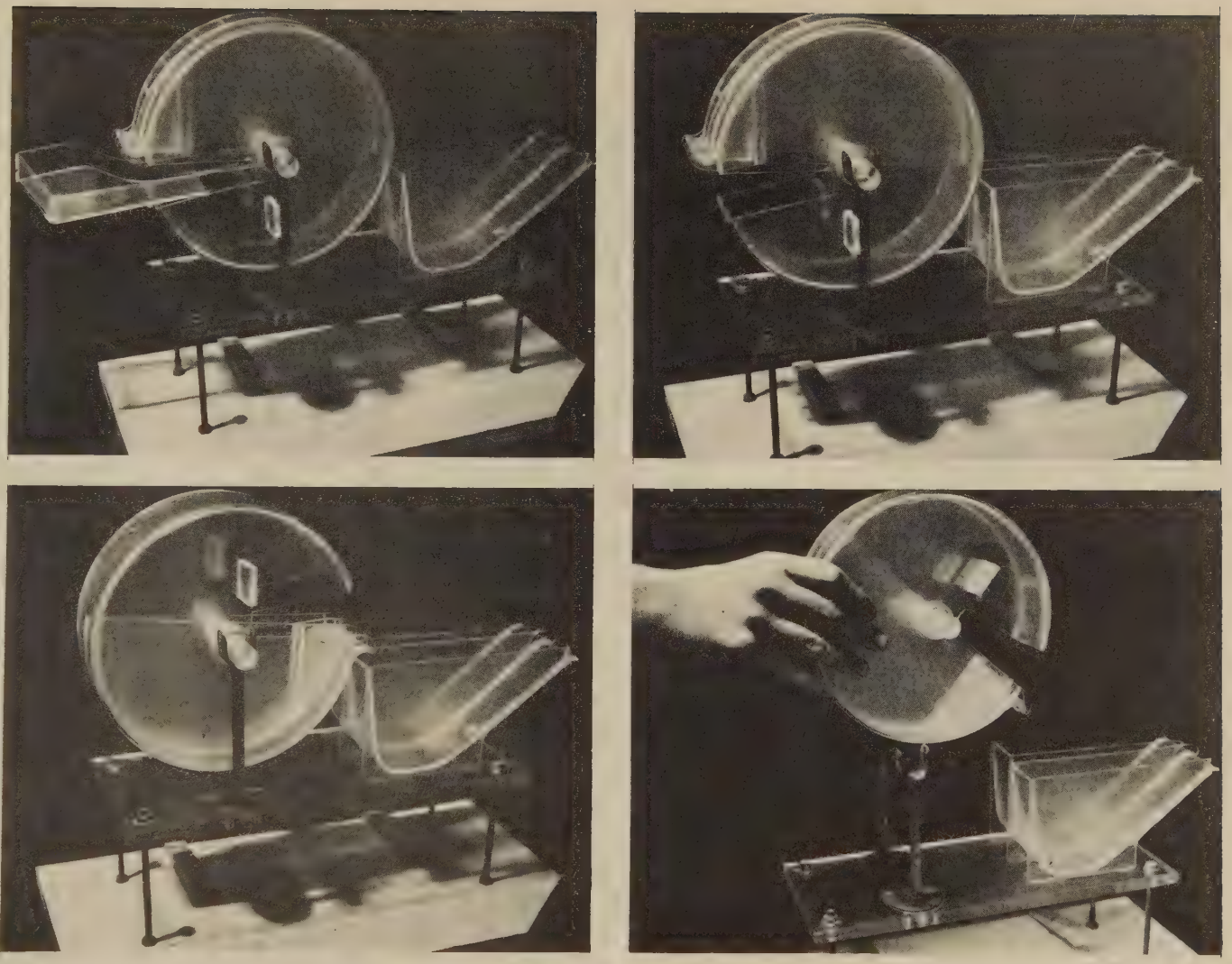


Figure 2.15. Folsom Plankton Splitter: a) septum inverted for pouring plankton sample into splitter; b) septum inverted for splitting; c) septum in place for divided sample; d) sample being poured into paired vessels. (Photos by K. Raymond, National Marine Fisheries Service, La Jolla, California.)

The total volume minus large organisms (Fig. 2.14) is the above volume minus the volume of large planktonic organisms such as large jellies or tunicates whose individual volumes exceed 5 ml.

The process of determining "wet" plankton volume by displacement is rather simple. The preserving liquid is removed from the plankton by pouring the sample through a draining cone, constructed of 0.333 mm Nitex. The plankton is retained in the cone until drainage of liquid from the cone diminishes to an occasional drop. The volume of the drained plankton is then determined by displacement in a graduated cylinder. Even after drainage, the sample will retain a considerable amount of interstitial liquid, usually between 30 to 40 percent of the total volume according to Ahlstrom and Thrailkill (1963). Usually no allowance is made for interstitial liquid; rather the total displacement volume of the drained "wet" sample is reported. The sample is returned to the original preserving liquid.

Additional information recorded on the plankton-volume data sheet (Fig. 2.14) includes station identification, the number of jars containing the sample, the proportions of fractioned samples, and the initials of the person who volumed the sample. Also, this data sheet is initialed by the sorter when the sample is checked out to be sorted (Section 2.2.3.).

2.2.2. Percentage of the Sample to be Sorted

It is recommended that total samples be sorted for fish eggs and larvae whenever possible, and that fractioning of samples be limited to those containing exceptionally large numbers of eggs and/or larvae or to samples with exceptionally large volumes of plankton material.

Several considerations should be weighed to determine whether an entire sample should be sorted for fish eggs and larvae, or only an aliquot. At times, it is desirable to sort only an aliquot for one category, usually eggs, and to sort the entire sample for the other, usually larvae. Sample size may or may not be a primary consideration for determining whether or not a sample should be aliquoted or completely sorted.

Eggs are often more abundant than larvae, and for many species are more aggregated so that occasionally, very large collections of eggs of the same species are obtained. For samples containing large numbers of eggs, it is usually advisable to split the sample into aliquots when sorting for eggs. The Folsom Splitter (Fig. 2.15) (McEwen, Johnson and Folsom, 1954) is a standard apparatus for dividing plankton samples into aliquot portions.

There are other considerations in sorting for larvae. One of the primary reasons for investigating larvae is to obtain information on the success of survival during the larval period. Such studies are postulated on the premise that the entire size range of larvae can be well sampled. Inasmuch as larger larvae may represent only a small percentage of the total number of larvae obtained of a species, it is usually necessary to sort entire samples in order to get an adequate representation of the larger-sized larvae.

2.2.3. Sorting Fish Eggs and Larvae from Plankton Samples

A sorter checks out a sample by initialing and dating the plankton-volume data sheet (Fig. 2.14). At this time a plankton sorter's work sheet (Fig. 2.16) is started which records the number of eggs and larvae removed from the sample. (Also refer to Section 2.2.4.).

Sorting is probably the step that requires the most time, e.g., one day per sample in the CalCOFI program. Before a sample is sorted, the preserving liquid (5% buffered Formalin) should be drained off because the formadehyde fumes, when breathed, can be irritating, even injurious to health. The sample can be sorted in a medium of fresh water or very weak Formalin solution. Precautions must be taken to prevent deterioration of the plankton material. If a sample has not been completely sorted during the day it was started, the unsorted plankton should be put back into 5% buffered Formalin during the night.

The best type of containers to use for sorting are small, e.g., Syracuse watch glasses or divided Petri dishes (Fig. 2.17.). They permit cleaner, i.e., more complete, sorting of fish eggs and larvae than is possible from larger containers. Borgorov's counting tray is also widely used (Newell and Newell, 1963).

Sorting is usually done under a dissecting microscope (Fig. 2.17) at a magnification of about ten times (10x ocular, 1x objective). It is inadvisable to try to sort samples with the unaided eye.

A small amount of the sample to be sorted is poured into one of the glass dishes. Its contents are examined closely under the microscope. All fish eggs and larvae are removed with pipettes and fine quality stainless steel forceps, counted, and placed in appropriately labelled dishes (Fig. 2.17.). The degree to which eggs and larvae are identified at this time will depend on the objectives of the survey and the training of the sorter (Sections 2.2.4., 2.2.5.). After the eggs and larvae have been sorted from a dish, its remaining contents are poured into a beaker labelled "Sorted". This process is repeated until the entire sample (or aliquot) has been sorted completely. The remaining plankton is then replaced in the original jar. The total number of fish eggs and larvae removed from the sample are recorded on the

TOTAL ORIGINAL VOLUME 68 SAMPLE NUMBER 6801-H-10.521 EC.

TOTAL ORIG. VOL. MINUS LG. ORG. 68 DATE COLLECTED I-18-68

FRACTIONED: YES (NO) SORTING:

PERCENTAGE _____ RIGHT DATE STARTED: 6-18-68

_____ LEFT TIME STARTED: 1:30 PM

PERCENTAGE VOLUME _____ PERSON SORTING DK

REMARKS: (Such as overall condition, etc.) PREDOMINANT TYPES OF PLANKTON:

Condition #4: oily, dirty, thinning necessary because of organism variety to be identified

Chaetognaths
Euphausiid
Lightenghous
Phyto - Radiolaria

_____ SARDINE EGGS

_____ SARDINE LARVAE

WHOLE

HEAD SECTIONS: _____ TAIL SECTIONS: _____

_____ ANCHOVY EGGS:

715 ANCHOVY LARVAE:

WHOLE 712

HEAD SECTIONS 3 TAIL SECTIONS 3

_____ SAURY EGGS

_____ SAURY LARVAE

_____ ETRUMEUS EGGS

_____ ETRUMEUS LARVAE

132 OTHER FISH EGGS

457 OTHER FISH LARVAE

WHOLE 457

HEAD SECTIONS _____ TAIL SECTIONS _____

Figure 2.16. Ichthyoplankton sorter's work sheet (From Kramer et al., 1972)

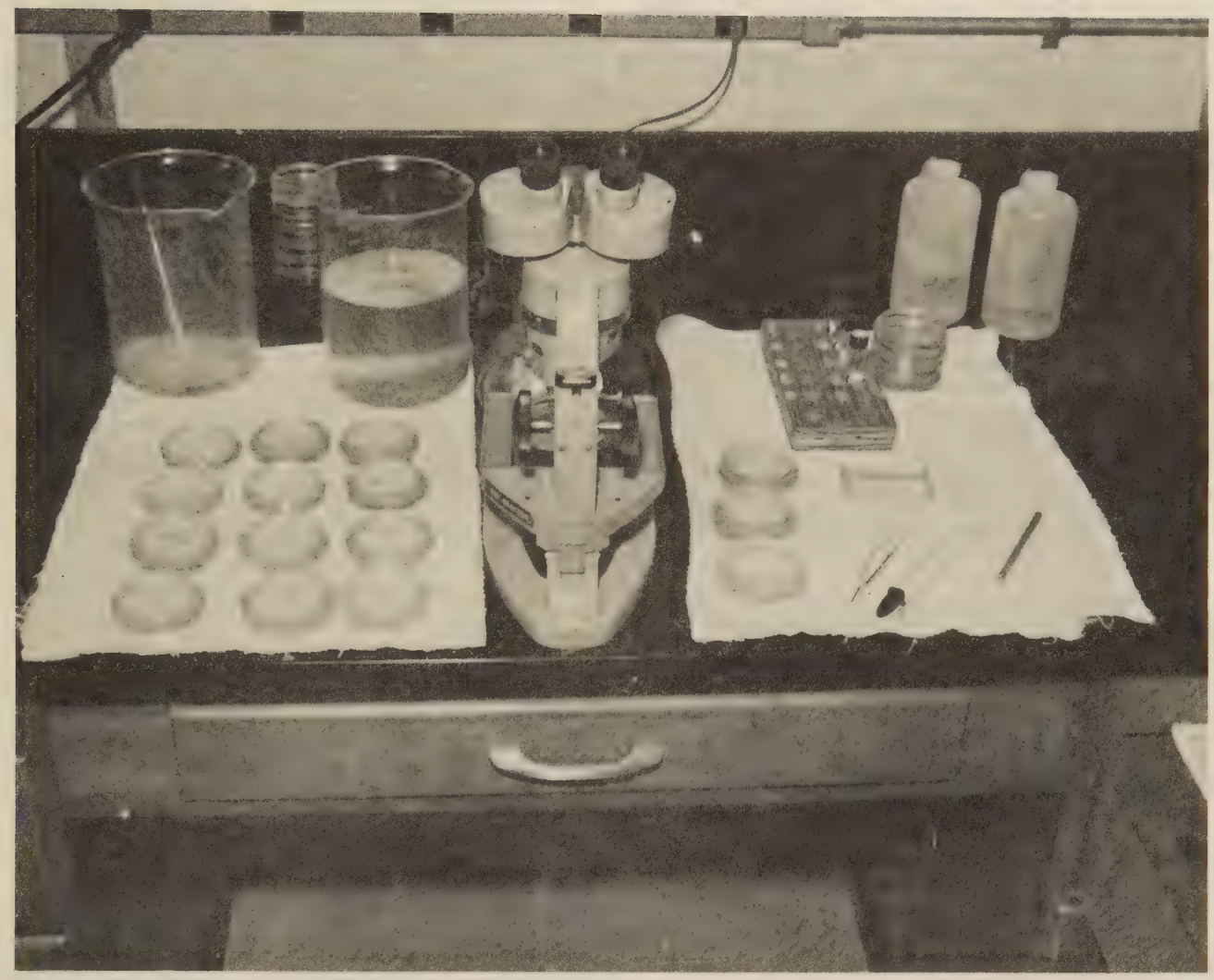


Figure 2.17. A laboratory sorting table. (From Kramer *et al.*, 1972)

CR 7203JD STA 77-51
DATE Mar 10 72 TIME 1246
TYPE TOW 1 m 505
TAXON OFE
NO. 35 ALIQ 100

Figure 2.18. Label used inside vials of sorted and identified fish eggs and larvae.

plankton sorter's work sheet (Fig. 2.16.). The eggs and larvae are then stored in separate appropriately labelled (Fig. 2.18.) vials. For most samples, 2-dram (about 7 ml) vials are quite satisfactory. The preservative added to the larvae is 3 percent buffered Formalin in tap water, which has proved to keep eggs and larvae in good condition indefinitely. The type of screw cap recommended for the 2-dram glass vials is plastic with a vinyl insert that is self-sealing when firmly tightened on the vial (Fig. 2.19). This screw cap is much preferred to corks, rubber stoppers, or screw caps lacking the vinyl insert because it retards evaporation.

The goal of sorting should be to remove 100% of the fish eggs and larvae. Thus, it is essential to establish checks on the completeness of sorting samples. This requires that either samples selected at random be completely re-sorted by another person or a system be devised for the routine rechecking by a second sorter of each sample sorted. Note: It is firmly recommended that the sorting of a sample for fish eggs and larvae be completed before sorting is commenced on other taxa in the sample. Sorting of the other taxa can be used as a means of rechecking for ichthyoplankton.

2.2.4. Preliminary Identification, Enumeration, and Measurement of Fish Eggs and Larvae

Technicians who sort fish eggs and larvae from other plankton constituents also can be trained to recognize and separate eggs and larvae of selected families of fishes such as clupeids, engraulids, scombrids, scomberesocids, carangids, etc. To identify eggs of some fishes, the technicians will have to make measurements of diamater of eggs and of their oil globules, if present. Technicians, i.e., sorters, can be readily taught to separate engraulid larvae from clupeid larvae; however, they should not be expected to make subtle distinctions among clupeid larvae, for example, or among engraulid larvae when several species of either family are represented in the collections. This is a task for ichthyoplanktologists.

If the eggs and larvae of selected species are identified at the sorting level, these species are enumerated and recorded (Fig. 2.16) separately with the remainder being placed in the categories OFE (other fish eggs) and OFL (other fish larvae).

Before bottling, larvae of the selected species may be measured and the lengths recorded on a tabulation sheet (Fig. 2.20). The larval length (standard length) is estimated to the nearest half millimeter. The larvae are measured under a microscope by passing each individual over a glass slide which has a transparent plastic rule taped beneath it.

After the data on preliminary identification, enumeration, and measurement have been recorded on the proper data sheets (Figs. 2.16, 2.20), the eggs and larvae are bottled for storage (Sect. 2.2.6.). An appropriate label (Fig. 2.18) is placed in each vial and the sample is kept together as a unit. Identifications made at the sorting level are rechecked by more highly trained "identifiers" to assure quality. After all the samples from a cruise have been sorted, a plankton sorters' master sheet (Fig. 2.21) is compiled.

Figure 2.19. Two-dram (7 ml) glass vials and plastic caps used for storing sorted and identified fish eggs and larvae. (From Kramer et al., 1972)

SPECIES: Anchovy CRUISE: 6801 STATION: 80.52 Leg 1 of 2
MEASURER: S. VanderBeek TOTAL LARVAE: (719) DIS: (3)

LENGTH (MM.)		TOTAL
2.0	1	1
2.5	1	1
3.0	IIII	4
3.5	𝍸 𝍸 𝍸 𝍸 IIII	24
4.0	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 I	61
4.5	𝍸 𝍸 𝍸 𝍸 𝍸 IIII	29
5.0	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 III	38
5.5	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 IIII	34
6.0	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸	30
6.5	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 II	37
7.0	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 IIII	69
7.5	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 IIII	54
8.0	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 IIII	64

Figure 2.20. Work sheet for tabulating measurements of fish larvae. (From Kramer, et al., 1972)

2.2.5. Final Identification of Fish Eggs and Larvae

The thoroughness with which collections of fish eggs and larvae are to be identified depends on the objectives of a survey and on personnel available for the work. The subject of identification is outside the scope of this manual. A bibliography of current research in this field is being prepared separately (FAO Fish.Circ.706)

If surveys for fish eggs and larvae are designed to deal with only one or a few species whose eggs and larvae can be readily identified, the identification problem discussed below does not exist and the task of identification can be assigned to easily trained technicians who also do the sorting and enumerating (Sections 2.2.3. and 2.2.4.).

Often the problem of identifying fish eggs and larvae is not this simple. In many groups it is difficult to distinguish between larvae of closely related species, as for example between the larvae of albacore tuna (Thunnus alalunga) and yellowfin tuna (Thunnus albacares) (Matsumoto et al., 1972) or between larvae of clupeid species belonging to the same genus, such as Sardinella (Fagetti, 1970), or between most species of engraulid larvae, or between herring (Clupea harengus) and sprat (Sprattus sprattus), etc. The problem is further complicated if all fish eggs and larvae are to be identified and enumerated. In most situations, the problem of the initial identification of fish eggs and larvae should be entrusted to competent ichthyoplanktologists.

The development stages of pelagic fish eggs and larvae have yet to be described in literature for a majority of marine fishes. The state of our knowledge is not as "primitive", however, as the above statement would seem to indicate. Early life history stages have been described for perhaps less than 10 percent of marine fishes, but, fortunately, these belong to a large number of marine fish families. By using information already available in literature, the majority of fish larvae (say 95 percent) can be identified to the family level. Scorpaenid larvae, for example, can be readily identified to the family level; life history stages have yet to be published, however, for most genera and species of Scorpaenidae. Hence, working out the life history stages of the various genera and species within a family becomes the primary task of the trained ichthyoplanktologist. The quality of literature dealing with the life history stages of fishes ranges from excellent to miserable, with a much higher percentage of poor contributions than good ones.

It is important that ichthyoplankton scientists be generalists as well as specialists, i.e., that they have the training and knowledge to identify fish larvae in general. To be a generalist, there is no alternative but to work on identification of all constituents (eggs and larvae) from total samples.

The egg and larval stages of some groups of fishes are particularly difficult to identify, such as those of scombrids (particularly tunas), clupeids, engraulids. Those of some other groups are difficult because they contain many genera and/or species, such as scorpaenids, serranids, carangids, labrids, paralepidids, etc. Hence, specialists are needed to work out the life history stages of all difficult groups. The work on any difficult group is facilitated if comparative material is available from other areas or oceans. There is a great need for coordination of such studies on a worldwide basis, in order to prevent duplication of effort, to

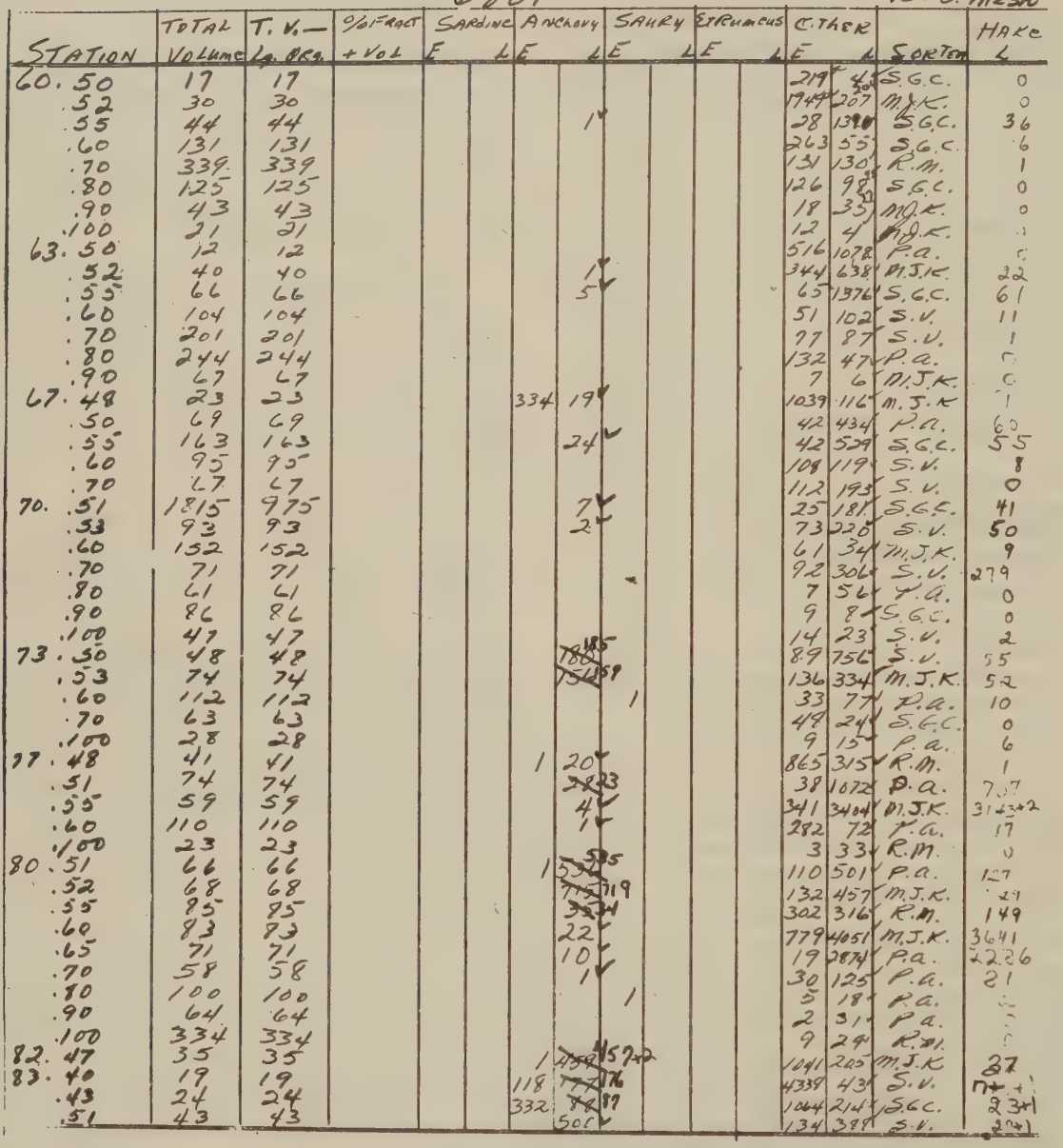


Figure 2.21. Ichthyoplankton sorters' master sheet. (From Kramer et al., 1972)

facilitate exchange of comparative materials, to stimulate work on neglected groups, and to encourage development of new techniques.

Not all kinds of fish eggs and larvae are difficult to identify to genus or species. In some groups of fishes, eggs and larval characters can be superior to adult characters for making definitive identifications to the genus or species level. Obvious examples would include myctophid larvae (Pertseva-Ostroumova, 1964; Moser and Ahlstrom, 1970; Moser and Ahlstrom, 1972), bathylagid eggs and larvae (Ahlstrom, 1971), and paralepidid larvae (Ege, 1930, 1953, 1957).

The most comprehensive series of collections of fish eggs and larvae has been made by the California Cooperative Oceanic Fisheries Investigations (CalCOFI). For working up CalCOFI collections, the decision was made at the initiation of the program

Cruise 6801 Station 80.52 Reg SHF 2,78 Total Larvae 1175

Anchovy L.	Sardine L.	J. mackerel	Pac. mackerel	Hake	Sebastodes	Anchovy E.	Sardine E.	Saury E.
719				29	326			
1,998.8				80.6	(S. flavidus - 28, S. jordani - 13) 906,3			

Citharichthys sp - 17 - 47.3
(C. stigmaeus - 1, C. sordidus - 2)

Goby - 5 - 13.9
Leuroglossus - 18 - 50.0
Paloneta - 1 - 2.8
Sciaenids - 10 - 27.8
Scorpaenichthys - 1 - 2.8

Protomyctophum - 3 - 8,3
S. leucopsarus - 45 - 125,1
Tarletonbeania - 1 - 2.8

Std. Total 3,266.5

Figure 2.22. Fish egg and larva identification sheet. (From Kramer et al., 1972)

to identify all constituents to at least the family level, and all common kinds to genus or species. When this is done, the person making the identifications records the data on a special identification sheet (Fig. 2.22.). The collections so treated to date are in the neighborhood of 30,000, a clear demonstration that this approach is feasible. This information has permitted a critical evaluation of the fishery resources of the California Current region. It should be noted that some species are dealt with in more detail than others. For example, larvae are measured routinely for only a few important species, i.e., Pacific sardine, northern anchovy, jack mackerel, Pacific mackerel and recently Pacific hake. Most of the CalCOFI egg and larva data have been put on tape for computer handling. Standardization of data is now done by computer, as well as summary tables, distribution charts, statistical tests, etc.

2.2.6. Bottling, Storing and Curating Identified Collections of Fish Eggs and Larvae

After a collection of fish eggs and larvae has been identified, it is usually inadvisable to try to bottle each category of fish eggs or larvae separately. Hence, a decision has to be made as to which categories should be bottled separately, and which kept together as a unit.

It is advisable to establish a reference collection of identified fish eggs and larvae which would include not only eggs and larvae of important species, but eggs and larvae of as many kinds of fishes as can be identified with certainty. Specimens selected for the reference collection are, of course, bottled separately. The original identification sheet (Fig. 2.22) should note all specimens of eggs and larvae that are separately bottled for placement in the reference collection. Within the reference collection, eggs and larvae of fishes belonging to the same family are kept together as a unit, arranged alphabetically by genus and species. A phylogenetic arrangement of families can be utilized, or an alphabetical arrangement of families. The former is the more convenient arrangement for trained ichthyologists, the latter for persons with less scientific background.

Collections of eggs and of larvae of species under major study should be bottled separately for ready retrieval if necessary. The eggs are usually "staged" and/or "aged"; larvae are measured. Sometimes food studies are made on gut contents of larvae, etc.

Eggs and larvae of species other than major species that are not separately bottled for the reference collection are best kept as units. Identified collections should be stored in an orderly manner, so that any given collection can be retrieved easily. For storing the 2-dram vials of eggs and larvae, a covered cardboard box having the dimensions length = 19 cm, width = 11.5 cm, depth = 7 cm is quite serviceable.

Samples stored in the above manner must be curated to prevent the samples from losing liquid by evaporation and eventually drying out. For ready retrieval of collections of fish eggs and larvae, such a storage system is most recommended. The curation time is well worth the effort. An alternative is to store samples in a bath of 3 to 5 percent Formalin. When so stored, the individual vials are usually stoppered with cotton. Perhaps the largest collection so curated is the Dana Collection (Carlesbergfondets at Charlottenlund Slot, Denmark), which utilizes 12-cm glass jars, 13.5 cm tall for storage of cotton-stoppered vials in a 5 percent Formalin bath. This is basically a museum storage technique, and cannot be recommended for collections that have to be consulted frequently.

2.3. Data Summarization

This section on data summarization considers only the recommended techniques for gathering the data collected on the ichthyoplankton survey for the estimation of spawning biomass. The analysis of errors in the process of gathering the data will be considered in Section 3, "Theoretical Considerations".

The end result of data summarization is to standardize the number of eggs or larvae in each plankton haul to the number under a unit area ($10m^2$ in this manual) of sea surface. This is a necessary prerequisite to estimating spawning biomass. Data forms used to assure good data summarization are shown in Fig. 2.23 and

SET I

1 OF 3

CRUISE NUMBER 6607-J
VESSEL JORDAN
DATES VII-9/28-66
CURRENT METERS 1179, 1181
MESH SIZE 0.505 μm Nitex

	STATION	ORDER OCCUP	TOWING TIME MIN	TOWING TIME SEC	TOWING TIME IN SECONDS	REV. OF CURRENT METER	REV./SEC	AVERAGE TANGENT	CALIB. FACT	C.F. X CONST	VOL H_2O STR (M^3)	COSINE OF AV TAN	WIRE OUT (METERS)	DEPTH OF TOW (METERS)	STAND HAUL FACTOR
1	60-50	1	4	00	240	1001	4.17	1.434	.147	.04455	41.6	.572	60	34.3	8.25
2	·52	2	7	31	451	1980	4.39	1.046	.146	.04427	81.7	.691	100	69.1	8.46
3	·55	3	11	45	705	3032	4.30	1.071	.146	.04427	125.1	.682	160	109.1	8.72
4	·60	4	21	34	1294	5655	4.37	1.027	.146	.04427	233.4	.698	300	209.4	8.97
5	·70	5	21	32	1292	(5388)	(4.17)	1.102	.147	.04455	(223.9)	.672	300	201.6	9.00
6	·80	6	21	42	1302	5260	4.04	1.125	.147	.04455	218.6	.664	300	199.2	9.11
7	·90	7	21	40	1300	4963	3.81	1.025	.147	.04455	205.8	.692	300	207.6	10.09
8	63-50	14	3	17	197	719	3.65	1.198	.147	.04455	29.9	.641	40	25.6	8.56
9	·52	13	7	43	463	1940	4.19	1.013	.147	.04455	80.6	.703	100	70.3	8.72
10	·55	12	21	37	1297	4838	3.73	.992	.147	.04455	201.0	.710	300	213.0	10.60
11															
12															

SET II

1 OF 3

CRUISE NUMBER 6607-J
VESSEL : JORDAN
DATES : VII-9/28-66
MESH SIZE : 0.505 μm Nitex

	STATION	VOL. H_2O STR. (M^3)	DISPLACEMENT VOL OF PLANKTON TAKEN IN TOW TOTAL (ML)	DISPLACEMENT VOL OF PLANKTON TAKEN IN TOW SM.ORG (ML)	PLANKTON VOLUME PER 1000 M^3 OF H_2O STR. TOTAL (ML)	PLANKTON VOLUME PER 1000 M^3 OF H_2O STR. SM.ORG (ML)
1	60.50	41.6	18		442	
2	·52	81.7	96	24	1170	290
3	·55	125.1	66		526	
4	·60	233.4	102		439	
5	·70	(223.9)	218		(974)	
6	·80	218.6	97		442	
7	·90	205.8	227		1102	
8	63·50	29.9	2		72	
9	·52	80.6	25		308	
10	·55	201.0	343		1704	
11						
12						

SET III

1 OF 3

CRUISE NUMBER 6607-J
VESSEL (S) : JORDAN
DATES : VII-9/28-66
MESH SIZE : 0.505 μm Nitex

	STATION	POSITION N. LAT	POSITION W. LONG.	DATE 1966	TIME (P.S.T) START	TIME (P.S.T) END	VOL H_2O STR (M^3)	DEPTH OF TOW (METERS)	PLANKTON VOLUME PER 1000 M^3 OF H_2O STR TOTAL (ML)	PLANKTON VOLUME PER 1000 M^3 OF H_2O STR SM.ORG (ML)
1	60.50	37 52.5	122 53.5	VII-9	1405	1409	42	0-34	442	
2	·52	37 58	123 01.1	-9	1500	1507	82	0-69	1170	290
3	·55	37 47	123 15	-9	1645	1657	125	0-109	526	
4	·60	37 37	123 37	-9	1915	1937	233	0-209	439	
5	·70	37 17	124 21	-10	0000	0022	(224)	0-202	(974)	
6	·80	36 56.5	125 04	-10	0455	0517	219	0-199	442	
7	·90	36 37	125 47	-10	1015	1037	206	0-208	1102	
8	63·50	37 23.3	122 22.8	-11	1530	1534	30	0-26	72	
9	·52	37 19	122 36	-11	1805	1813	81	0-70	308	
10	·55	37 13	122 50	-11	1125	1147	201	0-213	1704	
11										
12										

SET IV

1 OF 3

CRUISE NUMBER 6607-J
VESSEL (S) JORDAN
DATES VII-9/28-66
MESH SIZE 0.505 μm Nitex

	STATION	DATE 1966	MID-TIME OF TOW (P.S.T)	STAND HAUL FACTOR	PERCENT SORTED	S H F CORRECTED TO % SORTED
1	60.50	VII-9	1407	8.25	100%	—
2	·52	-9	1503	8.46		
3	·55	-9	1654	8.72		
4	·60	-9	1926	8.97		
5	·70	-10	0011	9.00		
6	·80	-10	0506	9.11		
7	·90	-10	1016	10.09		
8	63·50	-11	1531	8.56		
9	·52	-11	1408	8.72		
10	·55	-11	1136	10.60		
11						
12						

Figure 2.23. Forms used for data summarization. (Modified from Kramer et al., 1972)

CURRENT METER # 1179 Calib. 6/21/66 Hydraulics Lab By LJF

40 ft = 12.2 m

Secs.	Revs	Rev/SEC	M/Rev.
14.2	105	7.39	145
16.7	106	6.35	144
18.8	105	5.59	145
19.7	107	5.43	.142
21.7	106	4.88	.144
23.5	105	4.47	.145
25.8	107	4.15	.142
28.4	103	3.63	.148
29.4	105	3.57	.145
31.8	104	3.27	.147
34.0	103	3.03	.148
35.5	106	2.99	.144
38.0	104	2.74	.147

Figure 2.24. Record of tests for calibration of flowmeter #1179. (From Kramer et al., 1972)

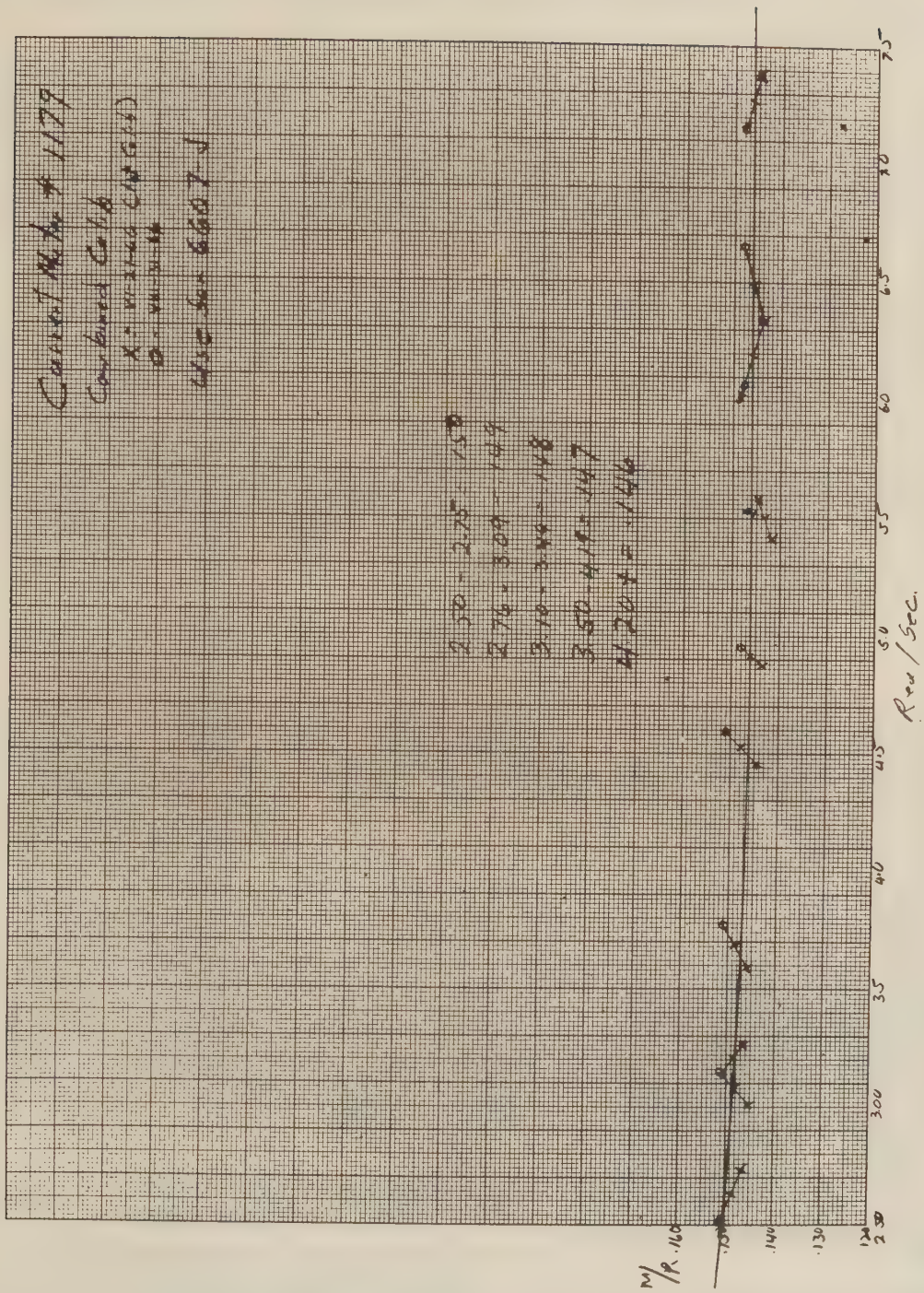


Figure 2.25. Flowmeter calibration curve. (From Kramer et al., 1972)

discussed in Section 2.3.4.

2.3.1. Flowmeter Calibration

To determine the standard haul factor for each tow requires that a calibrated flowmeter was placed in the mouth of each net and the flowmeter number and the revolutions made during each tow were accurately recorded (Item 12 on the plankton-tow data sheet, Fig. 2.8).

The calibration factor (f) is an expression of the number of meters the flowmeter travels during each revolution of the impeller (= m/rev). Different flowmeters are likely to differ from each other in this factor. The factor will not be the same for each towing speed for a single flowmeter. The same flowmeter may change its calibration factor gradually or may change it suddenly, if dropped, for example. For these reasons, each flowmeter to be used on a cruise is calibrated before each cruise, its performance is monitored during a cruise, and it is calibrated after each cruise. Spare flowmeters are taken on each cruise in case of loss or change in performance.

To calibrate a flowmeter, it is hauled or pushed through a measured distance of water at different speeds. The number of revolutions is recorded seperately for each test (Fig. 2.24). It is essential that uniform speed be maintained during each test. Calibration speeds should include speeds slow enough to define the friction point of each meter, and fast enough to bracket the range of speeds at which the flowmeter will be towed at sea.

The meters per revolution are then plotted against revolutions per second (Fig. 2.24) and form part of the calibration curve in Fig. 2.25. This graph, based on two calibration trials made before and after a cruise, provides an evaluation of the function of the flowmeter during the cruise. In this example, the calibration factors (f) are:

f_1 = 0.150 meters per revolution

f_2 = 0.149

f_3 = 0.148

f_4 = 0.147

f_5 = 0.146

Where the subscript number refers to individual ranges of speed:

1 = 2.50 to 2.75 revolutions per second

2 = 2.76 to 3.09

3 = 3.10 to 3.49

4 = 3.50 to 4.19

5 = 4.20

The latter value is good for all average towing speeds from 0.61 to at least 1.07 meters per second. The lowest usable average towing speed for this meter is 0.38 meters per second. Flowmeters are limited in performance at lower speeds due to proportionate increase in friction. The speed at which the meter stops is called the friction point. For the type of plankton tow recommended in this manual, flowmeters with friction points at or above 0.55 meters per second should be repaired or discarded.

2.3.2. Estimation of Missing Flowmeter Values

If a flowmeter value (number of revolutions) is missing from the plankton tow data sheet (Fig. 2.8), or if a recorded flowmeter value is obviously incorrect, it is possible to make an

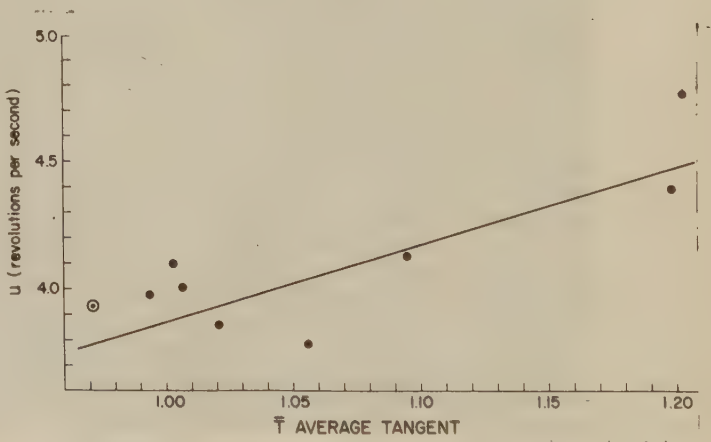


Figure 2.26. Regression line used to estimate a missing flowmeter reading (circled dot) for a particular flowmeter on a particular cruise, given average tangent for the tow in question and for other tows made with the same meter.

Table 2.1. Worksheet for calculation of the average tangent from the wire angles recorded each 10 meters of wire recovered (See Figure 2.8)

i	θ	tan θ
1	49°	1.1504
2	48	1.1106
3	47	1.0724
4	46	1.0355
5	47	1.0724
6	48	1.1106
7	48	1.1106
8	47	1.0724
9	47	1.0724
10	47	1.0724
11	46	1.0355
12	46	1.0355
13	48	1.1106
14	48	1.1106
15	46	1.0355
16	45	1.0000
17	47	1.0724
18	48	1.1106
19	48	1.1106
20	46	1.0355
21	45	1.0000
22	47	1.0724
23	49	1.1504
24	48	1.1106
25	46	1.0355
26	45	1.0000
27	46	1.0355
28	47	1.0724
29	45	1.0000
30	50	1.1918
SUM		32.2051
AVERAGE		1.0734

estimate of the missing value. The estimate is based on the average of all the wire angle readings made during the tow in question.

If a ship tows somewhat faster than usual, the wire angle will tend to be higher and the revolutions per second will also respond with an increase in rate. Consequently, a linear regression (Fig. 2.26) of the average tangent ($\bar{T}$)(Table 2.1 and Section 2.3.3.) and revolutions per second (u) based on other tows made by the same flowmeter may allow an adequate estimate of the number of revolutions for the tow in question. The precision of the estimate will be in part dependent on the number of tows taken with the same meter, and also on the technical quality of the tow records.

The least-squares method is used to calculate the regression line. The independent variable ($\bar{T}$) and the dependent variable (u) are listed in pairs (Table 2.2). The regression line, in this example, is described by the equation

$$u = 2.998\ \bar{T} + 0.874 \quad (r = 0.87\ ;\ r^2 = 75\%)$$

If the average tangent of the tow in question is 0.971 (Table 2.2), the estimated value of u is 3.79 revolutions per second.

2.3.3. Equation of Standardization

The basic equation of standardization is

$$C = 10\ (a^{-1}b^{-1}c\ d)$$

"C" is the number of eggs or larvae beneath a unit sea surface area (10 square meters in this case); "a" is the area of the mouth of the bongo net in square meters; "b" is the length of the tow path in meters; "c" is the number of eggs or larvae in the sample; and "d" is the maximum depth of tow in meters.

The value "a" is derived from the equation for a circle which is 60 cm in diameter (0.3 m radius);

$$a = \pi r^2$$
$$= 3.141\ \ (0.3)^2$$
$$= 0.2827$$

The value "b" is derived from the calibrated flowmeter:

$$b = f\ r$$

"f" is the calibration factor in meters per revolution (m/rev) for a given flowmeter at a given number of revolutions per second (Section 2.3.1.); and "r" is the number of revolutions of the flowmeter during the tow. For example, in Fig. 2.23, Set I, Line 6

$$b = 0.147\ (5260)$$
$$= 773$$

The value "d" is determined from the tow data by the equation;

$$d = W \cos\ (\tan^{-1}\ \bar{T})$$

"W" is the maximum length of wire out in meters (m); this is usually determined by a meter block in the towing winch system (Section 2.1.2.2.) and is recorded on the tow data sheet (Fig. 2.8); "$\bar{T}$" is the average tangent of the wire angle taken at 30 second intervals (Fig. 2.8) during the recovery phase of the plankton tow;

$$\bar{T} = \frac{1}{n} \sum_{i=1}^{n} \tan \theta$$

and "θ" is the angle at each reading of the wire from the plankton tow data sheet (Fig. 2.8, Table 2.1). For example, (Fig. 2.23, Set I, Line 6)

$$d = 300\ (0.664)$$
$$= 199$$

Thus, for this example (Fig. 2.23, Set I, Line 6) "a" is 0.2827; "b" is 773; and "d" is 199. If 50 larvae (c=50) were taken in the sample, the solution to the equation would be

$$C = 10\ \left(\frac{1}{0.2827} \times \frac{1}{773} \times 50 \times 199\right)$$
$$= 455\ \text{larvae per } 10\ m^2 \text{ sea surface.}$$

2.3.4. Data Forms Used for Standardization

The derivation of the equation of standardization (Section 2.3.3.) is not necessarily the same way the data become available or are needed. For sets of summary data sheets (Fig. 2.23) are used to assure the completeness and accuracy of organization of the data.

Set I (Fig. 2.23) summarizes the data from the plankton tow data sheet. Column 1 (in all four sets) is the station number. Column 2, which gives the order in which the station was occupied, comes from the "Captain's sheets" (Fig. 2.2). The order

Table 2.2. Worksheet for estimation of missing flowmeter readings by the method of least squares (regression).

TOW ORDER	AVERAGE TANGENT (T)	REVOLUTIONS PER SECOND (u)
1	1.243	4.76
2	0.971	3.79*
3	1.003	4.10
4	1.067	4.01
5	1.095	4.13
6	1.021	3.86
7	0.994	3.98
8	1.056	3.78
9	1.199	4.39

* estimated value

occupied is used to trace back faults in flowmeters (e.g., malfunction) and nets (e.g., clogging) which affect entire sequences of tows. Column 3 is the duration of tow (towing time) and is recorded in minutes and seconds on the tow data sheet (Fig. 2.8). Column 4 changes this value to seconds. The number of revolutions of the flowmeter is taken from the original tow data sheet and recorded in column 5. Column 6 lists the revolutions per second which is calculated by dividing the value in column 5 by the value in column 4. Revolutions per second is used to determine the calibration factor for the flowmeter (Section 2.3.1.). Column 6 is also monitored for high values to detect a cause of extrusion of small eggs and larvae. Column 7 is the average tangent derived from the original tow data sheet by converting the angle readings to tangents, adding them, and dividing by the number of readings (Table 2.1.). This value is rounded off to three places. It is used to determine the maximum depth of tow. The average tangent may also be used to estimate missing readings for a flowmeter which has been used for a series of tows on the same cruise (Sect. 2.3.2). Flowmeter calibration factors (Section 2.3.1) are recorded in column 8 and are read from the calibration curve (Fig. 2.25) derived for each flowmeter for each cruise at the rate of speed in column 6. In column 9 the mouth area of the net is multiplied by the calibration factor for use in determining the volume of water strained (filtered). In column 10 the volume of water strained is obtained by multiplying column 9, which is in m^3 per revolution, by column 5, which is in revolutions. In column 11 the cosine of the angle $\tan^{-1} \bar{T}$ is recorded for use in estimating the maximum depth of tow. This value provides a better estimate of the maximum depth of tow than the cosine of the angle with maximum wire out. Column 12, wire out is obtained from the original tow data sheet and is multiplied by the cosine of the angle $\tan^{-1} \bar{T}$ (column 11) to estimate the depth of tow (column 13). Column 14, the standard haul factor, is obtained by dividing column 13 by column 10 and multiplying the answer by 10. The standard haul factor multiplied by the number of eggs or larvae in the sample gives the number of eggs or larvae per 10 m^2 of sea surface.

Set II (Fig. 2.23) takes the information in Set I and the plankton sample displacement volume (Section 2.2.1.) and corrects the values to cubic centimeters of plankton per 1000m^3 of water strained. The values for volume of plankton may be used to estimate the sorting time for the sample.

Set III (Fig. 2.23) is the summary of plankton volumes in a form which may be published. It lists the actual station position from the "Captain's sheets" (Fig. 2.2) and the mid-time of tow from Set I. Since the normal tow is about 20 minutes long, the data from the original tow data sheet (Fig. 2.8) are only recorded to within 5 minute accuracy. This information is used primarily for the study and correction of the day-night difference in plankton volume and numbers of larvae. Other

Table 2.3 Basic data table for assembly of regional data to be used in making census estimates. Numbers represent sardine larvae per 10 m^2 sea surface (C_i, Section 2.4) for each station on each cruise. "0" indicates no larvae of this species were taken. "-0" indicates the station was not occupied.

STATION	CRUISE					
	01	02	04	05	07	10
3.1	5	356	83	3	9	0
3.2	-0	96	5	0	22	0
3.3	0	14	36	6	21	0
3.4	0	0	0	0	3	0
4.1	0	0	0	0	3	0
4.2	-0	0	6	3	35	3
4.3	-0	0	43	-	90	13
4.4	-0	-0	0	0	12	0
5.1	-0	0	0	3	16	0
5.2	3	49	3	6	0	5
5.3	0	0	4	0	3	0
5.4	0	0	4	0	0	0
NUMBER OF STATIONS OCCUPIED (N_i)	7	11	12	12	12	12
NUMBER OF STATIONS WITH LARVAE (N_L)	2	4	8	5	10	3
NUMBER OF LARVAE (ΣC)	8	515	184	21	214	21
ΣC^2	34	138,549	10,136	99	10,758	203
$\Sigma \ln C$	1.176	7.370	8.267	2.988	10.832	2.290
$\Sigma (\ln C)^2$	0.716	14.610	10.820	1.894	13.962	1.957

Table 2.4. Statistical summary table of regional data derived from basic data in Table 2.3. (Area factor = 3.29×10^9)

CRUISE	N	N_L	ΣC	$\bar{C}$	S_C	$\overline{\ln C}$	$S_{\ln C}$	F	P	$Lx10^9$
01	7	2	8	4.0	1.41	.588	.157	1.3	.286	4
02	11	4	515	128.75	155.18	1.843	.586	2.0	.364	154
04	12	8	184	23.00	29.04	1.033	.570	1.6	.667	50
05	12	5	21	4.20	1.64	.598	.165	1.2	.417	6
07	12	10	214	21.40	26.20	1.083	.498	1.4	.833	59
10	12	3	21	7.00	5.29	.763	.323	1.5	.250	6

Table 2.5. Statistical summary table of regional data derived from data in Tables 2.3. and 2.4.

SEASON	N	N_L	ΣC	$\bar{C}$	S_C	$\frac{n}{\ln C}$	$S_{\ln C}$	F	P	$Lx10^9$
Winter	18	6	523	87.17	136.37	1.424	.794	2.1	.333	96
Spring	24	13	205	15.77	24.16	1.645	.497	1.4	.542	28
Summer	12	10	214	21.40	26.20	1.083	.498	1.4	.833	59
Fall	12	3	21	7.00	5.29	.763	.323	1.5	.250	6
TOTAL										189

data columns are from Set I and Set II directly.

Set IV (Fig. 2.23) is used by the fish egg and larva "identifiers" to record sample data on the identification sheet (Fig. 2.22), e.g., standard haul factor, mid-time of tow, and percent of sample sorted.

2.4. Census Estimate (Larval Index)

To arrive at final census estimates, the spawning area and the survey season are divided into units which serve as pooled data areas. This allows a conservative estimate of precision and furnishes a concise summary of each year's spawning activity within each region. Data from the pooled area may furnish insight into changes in the allocation of sampling effort to maximize precision per unit cost for a target species (the primary species under study).

If it has been determined, for example, that the sample survey will be conducted over the four quarters of the year (1985) with two cruises each quarter in the spawning season and one cruise each quarter out of the normal spawning season, the stations close to each other may be grouped regionally and seasonally. If, for this example, the region of pooling (region #5) is 120 by 80 nautical miles and contains three lines of stations with four stations distributed along each line, the data for fish larvae may be assembled as shown in Table 2.3. At the base of each column, several sets of numbers have been calculated for analysis and inspection. They are the number of stations occupied (N), the number of stations at which sardine larvae occur (N_L), the total number of larvae taken in the region during the cruise

$$\Sigma C = \sum_{i=1}^{N} C_i$$

the sums of the squares of the numbers of larvae

$$\Sigma C^2 = \sum_{i=1}^{N} C_i^2$$

the sums of the logarithms of the numbers of larvae

$$\Sigma \ln C = \sum_{i=1}^{N} \ln C_i$$

and the sums of the squares of the logarithms of the numbers of larvae

$$\Sigma (\ln C)^2 = \sum_{i=1}^{N} (\ln C_i)^2$$

It is essential that these basic data are available for inspection so that questions arising in the interpretation can be traced to the original data.

These basic data (Table 2.3) are then summarized (Table 2.4). The original quantities N, N_L, and C are retained and the following quantities are calculated:

$$\overline{C} = \frac{\Sigma C}{N_L}$$

The mean number of larvae per positive station; and

$$S_C = [(\Sigma C^2 - N_L \overline{C}^2) / (N_L - 1)]^{\frac{1}{2}}$$

the standard deviation of the number of larvae per positive station; "$\overline{\ln C}$" the mean of the logs of larvae per positive station; "$S_{\ln C}$" the standard deviation of the logs of larvae per positive station; and "F" the error factor for the positive stations. "P" is the proportion of the stations which are positive (an estimate of the proportion of the sea surface underlain by larvae); and, "L", the larval index, is the regional census estimate of the number of larvae in this time period, which in the given example is

$$L = 3.29 \times 10^9 \ (P \ \overline{C})$$

where the factor 3.29×10^9 represents the number of 10 m^2 areas within the pooled region which in this case is 120 x 80 nautical miles.

Table 2.6. Total survey summary ($Lx10^9$) by years and regions.

YEAR	REGION									TOTAL
	1	2	3	4	5	6	7	8	9	
1978	84	321	204	439	250	131	1376	269	119	3193
1979	37	179	127	497	381	355	254	129	0	1959
1980	5	216	57	430	69	47	735	147	0	1706
1981	18	226	4	579	24	3	268	15	0	1137
1982	161	173	6	564	3	0	454	25	67	1453
1983	128	90	4	288	0	0	252	160	0	922
1984	187	120	70	374	181	5	406	152	11	1506
1985	36	35	6	99	189					

Table 2.7. Worksheet for comparing an independent biomass estimate with the larval index.

YEAR	BIOMASS ESTIMATE x 10^3 Tons	LARVAL INDEX x 10^9
1978	1336	3193
1979	850	1959
1980	586	1706
1981	424	1137
1982	562	1453
1983	380	922
1984	(593)*	1506

*estimate based on larval index

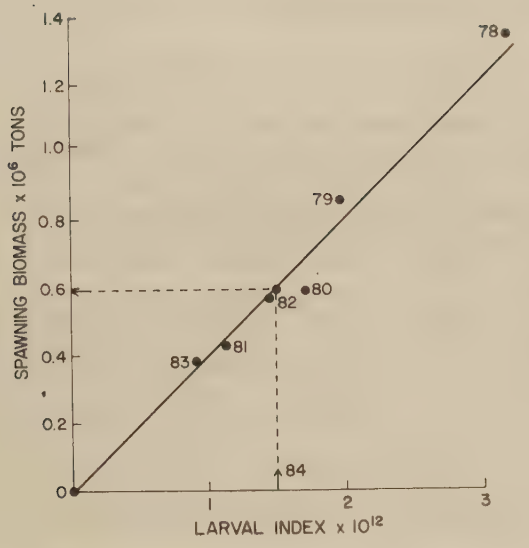


Figure 2.27. A scatter-plot of the relationship between spawning biomass and the larval abundance index. Numbers indicate years, e.g., 78=1978, etc.

Many of the factors displayed in Table 2.4 are used to assist in the evaluation of data. The cruises in this example do not represent equal periods of time, therefore, a further pooling of stations in time must be accomplished for the first two pairs of cruises. In Table 2.5, the two winter and spring cruises have been pooled and the regional census estimate of the number of larvae in the entire region in this time period has been obtained. This census estimate is an index of spawning intensity and coverage for this year in this region.

The larval index for region #5 in 1985 (total in Table 2.5) has been incorporated into the total survey summary in Table 2.6. This larval index can be used by itself to monitor changes in spawning biomass. The reliability of the larval index for such use is exemplified in Figure 1.2 when compared with two other independent estimates of biomass of the Pacific mackerel. The larval index can also be used in conjunction with other data to make instantaneous spawning biomass estimates. (Sections. 2.5, 3.6.)

2.5. Estimating the Spawning Biomass

One way to estimate the spawning biomass of the current population is by using the larval index of abundance. Other methods of making spawning biomass estimates from ichthyoplankton surveys are discussed in Section 3.6 along with problems involved in using them.

The method described here involves comparison of the larval index with another independent estimate of species abundance. This other estimate could be made, for example, by correlating the age composition of the stock with fishing effort and thus determining the number of fish in each age class as described by Murphy (1966). The larval index, when used in conjunction with Murphy's estimate, can make up for a generation of lag time and provide an estimate of the current spawning biomass.

A hypothetical example of this method of estimating biomass is provided in Table 2.7 and Figure 2.27. In this example, the larval index (total regional census estimate in Table 2.6.) is available from 1978 through 1984. The other independent estimate (Murphy, 1966) of fish biomass (Table 2.7) is available only through 1983. A linear relationship exists between these two estimates in the form of the equation:

$$B_s = 0.436\ L - 63.98\ (r = .989;\ r^2 = 98\%)$$

"B" is the spawning biomass of sardines, "L" is the larval index, "r" is the correlation coefficient, and "r^2" is the first estimate of the degree of dependence of spawning biomass on larval index. This equation provides a method of relating previous quantities of fish biomass with larva abundance in a way that can be used to estimate current spawning biomass. Thus by knowing the larval index for 1984 (1506×10^9), the spawning biomass (0.6×10^6 tons) for that year can be estimated.

Another example is provided in Figure 1.1. The sardine biomass was estimated from fishery data from 1940 to 1959 by the Murphy (1966) method. A larval index of abundance based on ichthyoplankton surveys was available from 1951 to 1969. The larval index was "calibrated" on the period of overlap (1951 to 1959) and it was then possible to make biomass estimates even though the fishery had essentially stopped.

3. Theoretical Considerations

Ideally, ichthyoplankton surveys conducted according to the methods presented in Section 2 would result in suitable estimates of the spawning biomass of any pelagic spawning fish. Unfortunately, even though the techniques may be standardized, the spawning behavior of pelagic organisms may vary widely enough to necessitate changes in techniques. For example, in individual cases it may be necessary to intensify the ichthyoplankton survey for a particular purpose or, conversely it may be possible to compromise some of the technical quality of the surveys and still obtain acceptable estimates of the spawning biomass. While the fundamental reason for this section is to provide information to determine individual requirements of standard ichthyoplankton surveys, it is hoped that this section will also serve two other purposes: 1) it should advise on ways in which surveys conducted with non-standard sampling techniques can be usefully related to the standard surveys; and 2) it should provide sufficient information on the sources and the magnitude of sampling error so that the survey specialist, with experience and the use of this manual and the research papers to which it refers, can make a contribution to the solution of continuing sampling problems.

3.1. Statistics

The present manual is not intended to be comprehensive with regard to statistical description and analysis of data. However, some subjects will be discussed to introduce the reader to particular problems encountered when sampling ichthyoplankton. Standard statistics texts should be consulted for the general concepts of statistical description, testing of hypotheses, and sampling design. For ichthyoplankton survey problems Southwood (1966) is recommended. A basic statistical description is given by Gulland (1966, 1969). Problems of biases and imprecision are treated in the UNESCO (1968) Monograph on Zooplankton Sampling. Sample design is treated specifically therein by Cassie (1968). The expense of marine plankton sampling and analysis may justify the services of a professional statistician for the planning of the data analysis.

3.1.1. Numbering Systems

The basic number system includes integers or whole numbers;

0, 1, 2, 3, 4, ... infinity (to an unlimited degree). These may also be extended to numbers on the negative side of zero to infinity. Between any two whole numbers there are fractions in this system which can be infinitely subdivided, e.g.:

0, 1, 2, 3, 4, ... infinity

2.1, 2.2, 2.3, 2.4,

2.21, 2.22, 2.23, 2.24.

Groups of numbers can also be elaborated to infinity as in the commonly referred to "orders of magnitude" system , e.g.:

0.1, 1, 10, 100, 1000, 10000, 100000, 1000000. . .

which in this example extends from one-tenth to one million by orders of magnitude. Since this system soon becomes unwieldy, an abbreviated system of "exponents" can be used:

10^{-1}	= 0.1	10^{3}	= 1000
10^{0}	= 1	10^{4}	= 10000
10^{1}	= 10	10^{5}	= 100000
10^{2}	= 100	10^{6}	= 1000000

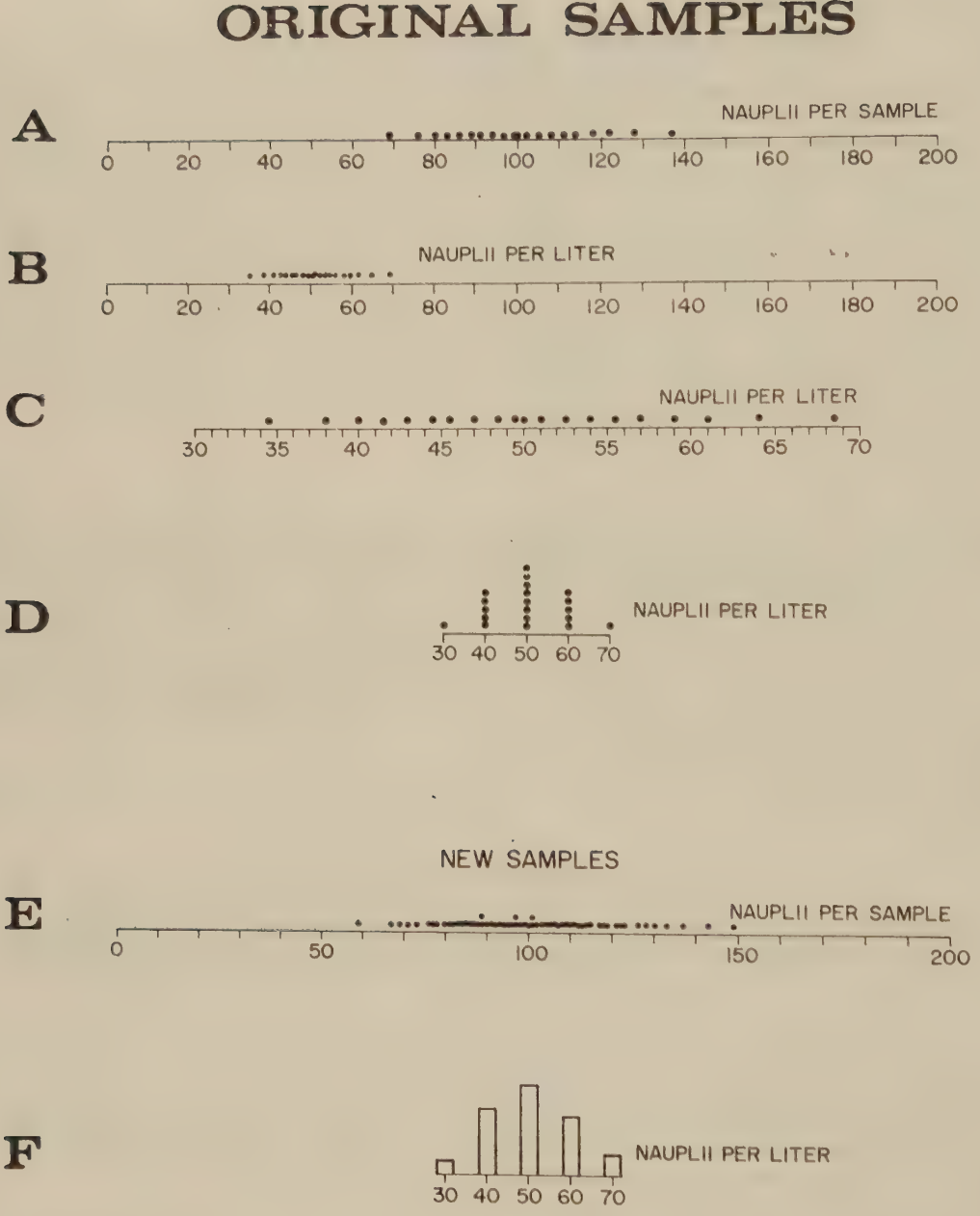


Figure 3.1. An example of sample data summaries. A) A graphic presentation of a number system from 0 to 200 with a dot at the position of each 2-liter sample count of nauplii. B) The 2-liter sample counts are represented as nauplii per liter. C) A segment of the scale has been expanded to the range of sample data expressed as nauplii per liter. D) The same sample array grouped in 10 nauplii per liter intervals, 30 (25-35), 40 (35-45), etc. E) New samples from the same population in nauplii per 2-liter sample. F) The sample array grouped in 10 nauplii per liter intervals. The length of each bar is proportional to the number of sample values within each interval.

These exponents can also be subdivided like fractions in the system of logarithms:

$10^{1.0}$	= 10	$10^{1.6}$	= 39.8		
$10^{1.1}$	= 12.6	$10^{1.7}$	= 50.1		
$10^{1.2}$	= 15.8	$10^{1.8}$	= 63.1		
$10^{1.3}$	= 20.0	$10^{1.9}$	= 79.4		
$10^{1.4}$	= 25.1	$10^{2.0}$	= 100.0		
$10^{1.5}$	= 31.6	$10^{2.7}$	= 501	$10^{3.7}$	= 5012

Approximations of logarithms of numbers and the number approximated by a logarithm may be found in books of mathematical tables. The precision of the approximation is controlled by the number of decimal places in the mantissa (the portion of the exponent following the decimal point). For example, the text table above may be used as one place table of anti-logarithms. The following is a two place table of logarithms, showing only the mantissae :

X	$\text{Log}_{10}X$	X	$\text{Log}_{10}X$
1	00	6	78
2	30	7	85
3	48	8	90
4	60	9	95
5	70		

The characteristic, or order of magnitude must be determined separately, as above. ($10^0 = 1$, $10^1 = 10$, $10^2 = 100$). The characteristic is the power of ten, and the mantissa is zero for all integer powers of ten. The mantissa is used for decimal fraction powers of ten. For example, $10^{0.70} = 5.012$, $10^{1.70} = 10 \times 5.012 = 50.12$, $10^{2.70} = 10^2 \times 5.012 = 501.2$, and $10^{3.70} = 10^3 \times 5.012 = 5012$. Note that the value X = 0 has no logarithm. Table 3.5 (below) is an abbreviated table of the mantissae. Tables of 4, 5 or 6 place logarithms and anti-logarithms are often available in books, and electronic calculators often have the capacity to generate 8 or 10 place equivalents.

3.1.1.1. Normal Distribution

Another form of number system is related to the distribution being sampled. For example, assume there is a well stirred brood tank of nauplii being reared to feed fish. Twenty successive two-liter samples are taken at random from the tank. Numbers (listed in descending order) of nauplii counted in each of the 20 samples are: 137, 128, 122, 118, 114, 111, 108, 105, 102, 100, 99, 97, 94, 91, 89, 86, 83, 80, 76, 69 (see also Fig. 3.1, line A). The ordered counts of nauplii per liter would be half the original numbers. On the fractional number scale they would be represented as in Fig. 3.1, line B. Only a portion of the fractional number system is necessary for this set of samples. On line C (Fig. 3.1), the samples are designated on a number scale which extends only from 30 to 70 nauplii per liter. Then all values are related to the nearest whole ten, 30, 40, 50, 60, 70, in line D (Fig. 3.1). Counts from 60 new samples of nauplii from the same tank are first represented by dots on the whole number scale (Fig. 3.1, line E) and then classified to "tens" in nauplii per liter (Fig. 3.1, line F).

There is a strong resemblance between the 20 samples and the 60 samples with regard to the central tendency of both distributions at 50 nauplii per liter and the absence of samples at 20 or less and 80 or more nauplii per liter. If the brood tank of nauplii had been completely separated into two liter units, there is reason to believe, on the basis of these two sets of samples, the general form of the distribution sample sizes in terms of nauplii per sample or nauplii per liter would be the same as the two sample sets illustrated. The only way to determine exactly how many nauplii were in the brood chamber would be to empty the chamber and count each nauplius: i.e., take a census. For many purposes the sample information was adequate and the census would contain little additional information. In many cases, like this, a census would be too expensive, unnecessary, or impossible. (For example, the census might kill the nauplii and render them unfit for fish food.) Also, during fast growth, the number of nauplii might change during a complete census but be stable for most purposes during a short sampling interval.

The example we have just examined was drawn from a "Normal" distribution. It was described by a set of samples which estimated two parameters; one for the position of the center on the number scale and one for the variation of the samples from this center. The central position is called the "arithmetic mean" or the "average" and the variation of the samples is called the "standard deviation." For the 20-sample series, the arithmetic mean (m) is determined by the equation:

$$m = \frac{X_1 + X_2 + X_3 + \ldots + X_{20}}{20}$$

which reads, the arithmetic mean equals the sum of the first number plus the second number plus the third number plus all the numbers up to and including the 20th number which then is divided by 20. It is usually abbreviated using the Greek letter "Σ" (Sigma) for the "sum of" and the smaller letter "i" to denote the subscript number for each "x" in the following manner:

$$m = \frac{1}{20}\sum_{i=1}^{20} X_i$$

which for this set of data is:

$$m = \frac{1}{20}\,(2009)$$

$$= 50.2 \text{ nauplii per liter}$$

It is conventional to call this the sample mean "m" and to denote the population mean it is intended to estimate by the Greek letter "μ" (Mu). If the brood tank held 4000 liters, then 2000, 2-liter samples could be taken and the equation for the population mean would be:

$$\mu = \left(\frac{1}{2000}\right)\left(\frac{1}{2}\right)\sum_{i=1}^{2000} X_i$$

$$= 50 \text{ nauplii per liter}$$

The second parameter which describes the "Normal" population is called the standard deviation "s_x". It refers to each measurement (x_i) to the population mean (μ). The sum of the differences between each measure and the sample mean will be zero; thus the standard deviation is calculated from the square of the values of the deviations of individual measurements, or in conventional shorthand:

$$s_x = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}\left(X_i - m\right)^2}$$

where s_x is the sample estimate of standard deviation of the variable "x", "n" is the sample size, and "m" is the sample estimate of the population mean as above.

The value "n-1" is used as a divisor, because if "n" is used, the sample standard deviation tends to underestimate the population standard deviation. This tendency is corrected by the use of "n-1" or "degrees of freedom" as the divisor rather than "n", the number of observations. The value s_x^2 is called the sample estimate of the variance of "x".

In the "Normal" population, the "range" (lowest number to the highest number) is infinite in theory, and sample estimates of the range can only increase with increasing sample sizes. For this reason, it is usual to seek instead a pair of values which encompass a stated proportion (say 95%) of the population. The sample standard deviation defined above may be used to estimate pairs of values which will encompass any proportion of the population or sample distribution. Tables for this

Table 3.1. Normal deviates (Z) and selected probabilities.

	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0.	.500	.460	.421	.382	.345	.309	.274	.242	.212	.184
1.	.159	.136	.155	.097	.081	.067	.055	.045	.036	.029
2.	.023	.018	.014	.011	.008	.006	.005	.004	.003	.002
3.	.001									

Table 3.2 Areas under the normal curve.

	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0.	.000	.040	.079	.118	.155	.192	.226	.258	.288	.316
1.	.341	.364	.385	.403	.419	.433	.445	.455	.464	.471
2.	.477	.482	.486	.489	.492	.494	.495	.497	.497	.498
3.	.499									

purpose are prepared for any situations which require great precision. Abbreviated tables for learning purposes are given in Tables 3.1 and 3.2 and these may suffice for all but the most demanding tests.

These tables (3.1 and 3.2) are complementary tabulations regarding the "normal" curve. The basic unit of the "normal" distribution is the difference between the observation and the population mean of all observations divided by the standard deviation. The unit is conventionally known by the letter "z" and calculated by the equation:

$$Z_i = \frac{X_i - \mu}{\sigma}$$

The letter "x_i" represents the variable under consideration, the Greek letter "μ" (Mu) represents the population mean as above, the Greek letter "σ" (Sigma) is the population standard deviation, and the letter "Z_i" is the number of standard deviation units "x_i" is from the population mean. In Table 3.1, the "Z" value of 0.0 represents the arithmetic mean of the distribution. The value at the intercept of the 0. row and the .0 column is .500 which is interpreted as meaning that half the sample values are above and half the sample values are below the arithmetic mean. The intercept of the 1. row and the .3 column is .097. This is read as 9.7% of all sample values

are above the arithmetic mean plus 1.3 standard deviations and 9.7% of all sample values are below the arithmetic mean minus 1.3 standard deviations. In the complementary table 3.2, the intercept of the 1. row and the .3 column is .403. This is read that 40.3% of all sample values are between the arithmetic mean and the arithmetic mean plus 1.3 standard deviations and 40.3 % of all sample values are between the arithmetic mean and the arithmetic mean minus 1.3 standard deviations. Note that the values of the intercept of 1. row and the .3 column are .097 in Table 3.1 and .403 in table 3.2 and these values add to .500. All similar intercepts in the two tables are complementary thus either table alone would suffice for both types of expression. Lastly, asymmetric statements of probability may also be made from these tables. For example, the value at the intercept of row 1. and column .6 in table 3.2 is .445 and this may be stated: 44.5% of all sample values lie between the arithmetic mean and the arithmetic mean plus 1.6 standard deviations. Since we know that 50% of all sample values lie below the sample mean, this means 94.5% of all sample values lie below the arithmetic mean plus 1.6 standard deviations.

From the nauplii example (Table 3.3), the mean number of nauplii per liter is 50.225, the standard deviation is 8.91, the range, 34.5 to 68.5 nauplii per liter, and range of deviations extends from 15.725 nauplii per liter below the mean to 18.275 nauplii per liter above the mean. Expressed in standard deviation units the lowest value is 1.76 standard deviations below the mean and the highest value is 2,05 standard deviations above the mean. This is not an unreasonable asymmetry for such a small sample. One is justified for suspecting that these 20 samples have not explored the entire range of the 2000 samples possible from this tank. One sample standard deviation below the mean is 50.225 - 8.91 or 41.315. Sample values x_1, x_2 and x_3 are lower than this value. One sample standard deviation above the mean is 50.255 + 8.91 or 59.135. Similarly, sample values x_{18}, x_{19}, and x_{20} lie above this value. Thus, 3/20 or 15% of the values lie more than one standard deviation below the mean and 3/20 or 15% of the values lie more than one standard deviation above the mean. This means that 70% of all samples lie between the one standard deviation limits about the mean.

Table 3.1 at 1.0 z, gives the population value for this as .159 or 15.9% of sample values are more than one standard deviation above the mean and 15.9% are more than one standard deviation below the mean. This means that 100% - 15.9% or 68.2% are within ± one standard deviation unit of the mean. As stated above, Tables 3.1 and 3.2 are complementary in the sense that one gives the proportion of values likely to be outside set standard deviation units, while the other gives the proportion of values likely to be within given standard deviation unit boundaries. One should become completely familiar with both methods of presentation. Particular attention should be paid the value 1.96 standard deviations above and below the mean, because this means that 2.5% of all sample values lie outside the upper and lower bounds or 95% of the values lie within these bounds. This should be committed to memory for statistical work since estimation of the chance of being wrong only once in 20 times (95%) is a common statistical practice.

Thus, of the number systems mentioned so far, the whole number system, the fractional number system, the logarithmic system, and the normal system, the latter is the only system which is defined by a population of counts or measurements with the central position determined by the arithmetic mean and a number in "z" units of standard deviations above and below the arithmetic mean. One property of the normal system should be noted: the sample standard deviation is independent of the sample mean and thus the two parameters are independent measures of the population. It is important to realize that these constraints are not often satisfied in the natural distribution of animal populations in the sea or elsewhere, although on occasion parts of the sea may approach a state of being mixed somewhat like the tank of nauplii in the example.

Another example of the normal distribution and a simple graphic method for testing its utility, may be seen from the data of Fraser (1969) regarding the degree of penetration of oceanic plankton into the North Sea (Table3.4; Fig. 3.2.). For this example the North Sea extreme position of the oceanic plankton was recorded for 32 of the 45 years between 1920 and 1965. These positions were grouped into 30-mile categories in Table 3.4. At the right margin of the table the individual

Table 3.3. Ordered set of data from a normal distribution of samples of nauplii (an example).

SAMPLE	NAUPLII PER LITER	DEVIATION FROM MEAN
x_1	34.5	-15.725
x_2	38	-12.225
x_3	40	-10.225
x_4	41.5	-8.725
x_5	43	-7.225
x_6	44.5	-5.725
x_7	45.5	-4.725
x_8	47	-3.225
x_9	48.5	-1.725
x_{10}	49.5	-0.725
x_{11}	50	-0.225
x_{12}	51	+0.775
x_{13}	52.5	+2.275
x_{14}	54	+3.775
x_{15}	55.5	+5.275
x_{16}	57	+6.775
x_{17}	59	+8.775
x_{18}	61	+10.775
x_{19}	64	+13.775
x_{20}	68.5	+18.275
	$\sum x_i = 1004.05$	$\sum (x_i - m) = 0.0$
	$m = 50.225$	$md = 0.0$
		$\sum (x_i - m)^2 = 1509.2375$
		$s = 8.91$

Table 3.4. Penetration of oceanic plankton into the North Sea (after Fraser, 1969).

N°	n.mi.	YEAR									N	%	CUMULATIVE %
60.0	0	37									1	2.9	2.9
59.5	30												
59.0	60	32									1	2.9	5.7
58.5	90	51									1	2.9	8.6
58.0	120	26	59	60							3	8.6	17.1
57.5	150	20	23	24	25	30	35	36	57	63	9	25.7	42.9
57.0	180	21	33	48	50	58	61	62	65		8	22.9	65.7
56.5	210	28	52	53	55	56	64				6	17.1	82.9
56.0	240	38	39	49	54						4	11.4	94.3
55.5	270												
55.0	300	47									1	2.9	97.1
54.5	330												
54.0	360	46									1	2.9	100.0

group percentages and the cumulative group percentages are listed. Special graph paper, called probability paper, which has the cumulative percentage axis adjusted so that a "normal" distribution would be represented as a straight line, was then used. The axis is numbered from 0.01% to 99.99% The cumulative percentages from 17.1% to 97.1% from Table 3.4 were plotted in position (Fig. 3.2). The fact that these points are suitably close to a straight line is consistent with a hypothesis that the tendency of oceanic plankton to penetrate the North Sea is a "normally" distributed variable. The utility of this graphic method is that data sets can be simultaneously compared with respect to the "normality" of the distributions, the similarity of the means, and the standard deviations.

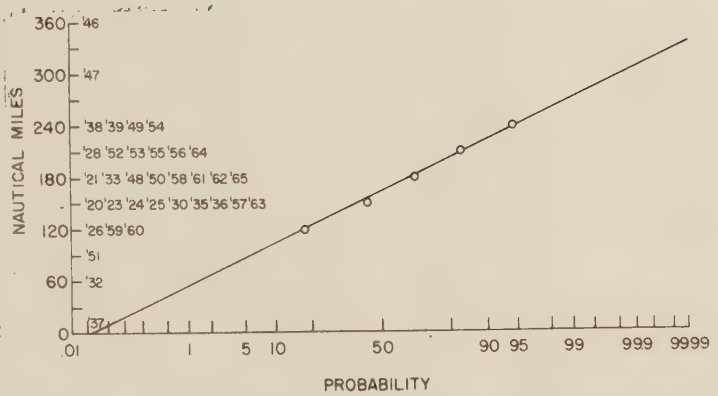


Figure 3.2. Penetration of oceanic plankton into the North Sea. The data in Table 3.4 have been plotted on normal probability paper by use of the cumulative percent frequency of Table 3.4 as the abscissa and the nautical miles of penetration recorded by planktologists as the ordinate. (After Fraser, 1969)

3.1.1.2. Lognormal Distribution

In the lognormal system (Aitchison and Brown, 1957), the sample values vary by a series of proportions of the central value rather than by a constant amount from the mean as in the Normal distribution. The uses of the central measure and the standard deviation are the same: the difference is that the equations are

$$\log g = \frac{\log X_1 + \log X_2 + \log X_3 + \ldots + \log X_n}{n}$$

or, as before

$$\log g = \frac{1}{n} \sum_{i=1}^{n} \log X_i$$

with the difference that the value "g" is the geometric mean of the set of numbers rather than the arithmetic mean (m) in the cases above. The sample standard deviation is similarly determined as above but now refers to a proportion to be multiplied or divided by the geometric mean to determine the distribution of values.

To save the trouble of calculating the geometric mean and the ratios of all values and the deviations of the ratios from unity, it is conventional to convert all values to logs (any base) and proceed as in the normal distribution. Table 3.5 provides a handy method for converting measurements or counts to logs. As stated above, if the numbers to be so transformed are between 1 and 10, the value in the table is used in a base 10 log transformation. If the numbers are between 10 and 100, the number in the table is preceded by the value "1.", and so on for other orders of magnitude. The conventional notation may also be used for the sample standard deviation of the log of the geometric mean:

$$s_{\log X} = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (\log g - \log X_i)^2}$$

It is important to note two limitations on the use of the lognormal distribution: it must be demonstrated in each case (as in the normal distribution) that the variance is independent of the mean; and, "Lognormal theory cannot be applied directly to any sample which contains a zero value." (Aitchison and Brown, 1969, p. 94). For example, in the equation for the mean there is no logarithmic equivalent for x_i = '0':

Table 3.5. Three place mantissae for base ten logarithms.

	0	1	2	3	4	5	6	7	8	9
1	000	041	079	114	146	176	204	230	255	279
2	301	322	342	352	380	398	415	431	447	462
3	477	491	505	519	532	544	556	568	580	591
4	602	613	623	634	644	653	663	672	681	690
5	699	708	716	724	732	740	748	756	763	771
6	778	785	792	799	806	813	820	826	833	839
7	845	851	857	863	869	875	881	887	892	898
8	903	909	914	919	924	929	935	940	945	949
9	954	959	964	969	973	978	982	987	991	996

similarly in the equation for the standard deviation, the expression (log g - log x_i) is equivalent to division by '0' when x_i = '0'.

Users of this manual must assure themselves that in each case where lognormal analyses are used, sufficient care is taken that the assumptions critical to these analyses are examined. Bagenal (1955) lists several misuses of the lognormal distribution in marine plankton sampling and also describes the proper use of the method. In particular, the use of logarithmic transformations with the natural base "e" is recommended for sample statistics instead of the base '10' logarithm because of the ease with which unbiased arithmetic means may be derived from the mean and variance of ln x_i. The equation for the mean and variance are as above with the transformed variate.

3.1.1.3. Negative Binomial Distribution

In the Normal and lognormal distributions the variance is independent of the mean. In the negative binomial distribution, the variance is related to the mean in the following manner:

$$s^2 = m + \frac{m^2}{k}$$

A theoretical discussion of the properties of the negative binomial is beyond the scope of the manual (See Anscombe, 1949; Fisher, 1941). It has been found to be useful in the study of trawl catches of fish (Taylor, 1953).

One goal of statistical analysis, is to minimize the money and effort expended to obtain and interpret data. For the purpose stated for this manual, i.e., to monitor important changes in the spawning biomass of fish through ichthyoplankton surveys, one can identify the level of sampling effort necessary to achieve suitable separation of important differences. For example, if one wishes to distinguish between years differing by 25%, and if one assumes that the negative binomial is the most appropriate distribution model for ichthyoplankton, the following equation (Southwood, 1966, p. 20) will allow a first approximation of the number of samples needed to achieve the monitoring goal precision (25%):

$$N = \frac{\frac{1}{m} + \frac{1}{k}}{D^2}$$

"N" is the number of samples required, "m" is the sample mean of the number of eggs or larvae per unit area, "k" is the dispersion parameter of the negative binomial distribution, and "D" is the percentage level of difference required to discriminate between important and unimportant changes. For example, if we use the value for sardine eggs within one day of spawning (Smith, 1973) of about 100 eggs per 10 m^2 for "m", and a "k" of 0.014, and a "D" of 0.25 (25% difference) the number of samples required is:

$$N = \frac{\frac{1}{100} + \frac{1}{0.014}}{(.25)^2} = \frac{71.43}{0.0625} = 1143$$

The value of "D" is selected arbitrarily by the researcher to meet the goals of the program with respect to detection of important differences. The value of "k" may be estimated in various ways (Southwood, 1966) but the simplest estimator is:

$$k = \frac{m^2}{s^2 - m} \qquad k = \frac{10,000}{714,386-100}$$

As above, "m" is the arithmetic mean, and "s^2" is the variance or the square of the standard deviation "s". These sample values include the "O" samples, if any, in the preliminary samples.

Table 3.6. Estimations of the number of samples necessary to obtain certain levels of precision (D) for varying degrees of patchiness (k).

k	D			
	.05	.10	.25	.50
0.01	40004	10001	1600	400
0.02	20004	5001	800	200
0.05	8004	2001	320	80
0.10	4004	1001	160	40
0.20	2004	501	80	20
0.50	804	201	32	8
1.00	404	101	16	4
2.00	204	51	8	2

Table 3.6 represents a calculation of the number of samples required to discriminate between samples at levels of difference from 5% (0.05) to 50% (0.50) when the value of "k" varies from 0.01 to 2. The mean number of eggs or larvae per sample is arbitrarily fixed at 100 for this table.

3.1.2. Data Comparisons

In addition to the preparation of data summaries (see Section 2.3.) for practical evaluation of spawning biomass estimates, it is often necessary to examine certain statistical properties of the data set. For example, the incidence of a single large sample, during any single year of a 10-year sampling program, may lead one to believe quite falsely that the year has been unusual. It has been emphasized that the arithmetic mean is less and less efficient as the distribution of sample sizes gets skewed. Certain consequences of this fact may be lessened by understanding the distribution of spawning products (See Section 3.4. below) and presenting data in such a way as to expose rare large samples.

In Table 3.7 are three characteristic number distributions illustrating one "normal" distribution and two "lognormal" distributions with different degrees of standard deviation of the logs. All three distributions are centered at about 100, i.e., about an equal number of observations exceed 100 as are lower than 100. We will briefly examine some of the statistical properties of these three number sets.

Table 3.7. Three characteristic number distributions.

Normal		Log Normal "A"		Log Normal "B"	
149	99	479	96	17785	100
143	98	380	93	9813	89
137	97	339	91	5012	79
133	97	302	89	3981	71
130	96	269	85	2815	66
128	95	263	83	2239	63
126	94	246	81	1778	56
123	93	229	79	1413	50
122	92	214	76	1122	45
121	81	195	74	1097	40
119	90	191	72	891	35
118	89	178	69	708	32
117	89	166	63	631	28
115	88	162	62	562	25
114	87	159	58	501	22
113	86	148	56	447	20
112	85	145	55	398	18
111	84	141	52	355	14
110	83	138	51	316	13
109	82	135	50	282	11
108	81	132	47	269	10
107	80	129	45	251	9
106	78	123	43	224	8
105	77	120	41	200	6
104	76	118	39	178	4
103	73	115	37	159	4
102	71	110	32	148	3
101	69	107	28	141	2
101	67	105	20	126	1
100	59	102	15	112	0.7

	Normal	Lognormal "A"	Lognormal "B"
m (arithmetic mean)	100.55	123.70	899.70
s_x (standard deviation)	19.58	92.39	2642.71
Coefficient of variation	19%	75%	294%
ln g	1.99	1.99	2.01
s_g (standard deviation)	0.09	0.31	9.98
g (geometric mean)	98.67	96.70	102.21

In Table 3.8 we have divided the original normal number set into classes. In column "A", the class boundaries are 57.5 to 62.5, 62.5 to 67.5, and so on, for 19 classes. The representative value for each class, or "point" is 60, 65, 70, . . . 145. In Column "B" the class boundaries are 55 to 65, 65 to 75, and so on, for 10 classes, and the class points are 60, 70, 80, . . ., 150. In Column "C" the class boundaries are 50 to 70, 70 to 90, and so on for 5 classes. The point values are 60, 80, . . ., 140. In Column "D" the class boundaries are 40 to 60, 60 to 80, and so on for 6 classes. We find that the parameter estimates from these classified data sets differ very little from the population mean, 100.55, and the population standard deviation,19.58. In each case, the "point" of the class was multiplied by the frequency value for the class, the results were summed and divided by the summed frequency. It is likely, the "C" or "D" classifications would be more easily understood as a normal frequency distribution, by inspection, than any of the other classified distributions or the complete data set itself (Table 3.7).

Table 3.8. Sample frequency distributions with varying class boundaries and points for the normal distribution in Table 3.7.

POINT	POINT ± 2.5	POINT ± 5	POINT ± 10	POINT ± 10	UNGROUPED
50					
55				1	
60	1	1	3		
65	1				
70	2	4		8	
75	3				
80	4	8	17		
85	5				
90	6	12		22	
95	6				
100	6	12	22		
105	5				
110	5	10		19	
115	4				
120	4	6	14		
125	2				
130	2	4		8	
135	2				
140	0	2	4		
145	1				
150	1	1		2	
m	100.58	100.50	99.67	100.33	100.55
s	19.79	19.43	20.00	20.99	19.58

In a similar manner, the logarithmic distributions may be summarized to inspect the frequency distribution. In Table 3.9 one may see the two lognormal number sets summarized by classes. The classes increase by multiples of four (any multiple may be used) and the class boundaries are 0.25 to 1, 1 to 4, 4 to 16, and so on for nine classes, and the class points are the geometric means of the class boundaries, 0.5, 2, 8, 32, 128, . . . , 3276. One must note that the arithmetic means are not particularly precise estimates of the population mean, but they are likely no more imprecise than sample estimates of 10 or 20 from distributions of this kind. Still the general lognormal appearance of the data is emphasized and the lognormal and arithmetic parameters of the mean and standard deviations are unbiased.

It is important to become familiar with the distributions for each planktonic stage for each species of fish. In this way, errors in judgment may be avoided or minimized.

Some examples from the CalCOFI program may serve to illustrate some kinds of statistical distributions of ichthyoplankton. For example, the fundamental sample distribution of sardine eggs has not changed markedly, even though the population

Table 3.9. Sample frequency distributions with even logarithmic class boundaries and log-normal frequencies (Table. 3.7)

CLASS BOUNDARY			LOG	LOG-NORMAL	LOG-NORMAL
LOWER	UPPER	MID-POINT	MID-POINT	A	B
.25	1	0.5	-0.30		1
1	4	2	0.30		3
4	16	8	0.90	1	9
16	64	32	1.51	17	12
64	256	128	2.11	36	14
256	1024	512	2.71	6	11
1024	4096	2048	3.31		7
4096	16384	8192	3.91		2
16384	65536	32768	4.52		1
			m	137.20	1189.57
			s	132.33	4425.54

has decreased 20-fold (Table 3.10). The scale of sample has been different with each decade, but the basic distribution of the all-important large samples remains proportionate. The lower end of the distribution is a sampling threshold and it appears to be essentially lognormal as in 1931-32 but the "unit" sample (one egg per sample) appears to be a pool of all areas less than the upper boundary of the smallest sample size class. Thus the high-speed sampler of 1950 with a small mouth opening, could only represent unit samples for all areas with an average of less than 256 eggs per 10 m^2. One may also note the remarkable stability of the standard deviation and the negative binomial "k".

The sensitivity of the sample frequency distribution is illustrated by the spawning cycle of the hake (Table 3.11) and the respective samples of eggs and larvae from a typical hake season, December 1954 through April, 1955. This is probably the most intense pattern exhibited by ichthyoplankton in the California Current. This will likely be explained by the fecundity of the individual females, the habit of spawning below the mixed layer of the ocean, and the long persistence of eggs and larvae in the colder waters.

One is often confronted with questions like "Are samples of type "A" comparable with samples of type "B"?" Of the statistical tests, few are as widely useful in ichthyoplankton sampling as the Kolmogorov-Smirnov Test (Tate and Clelland, 1957, p. 62). Two examples are provided here; one in which the samples are drawn from different distributions and one in which the samples are drawn from the same distribution.

The first example is drawn from a test which asks the question "Are anchovy eggs the same diameter in August of 1972 as they were in February of 1972?" In Table 3.12, the midpoints of the frequency classes of the diameters of the eggs are listed as fractions of a millimeter from 0.5643 to 0.8550 and the number of eggs measured at each size is in the second column. The next column lists the percent each category is of the total and the last column is the cumulative sum of the percents for the month of February.

Table 3.10. Sample frequency distributions of sardine egg samples at different levels of population taken with different plankton tows.

CLASS BOUNDARY ($N/10m^2$)			GEAR TYPE AND YEAR OF SAMPLES				
LOWER	UPPER	MID-POINT	CF&G 1931-32	1-Meter 1941	1/2-Meter 1941	High-Speed 1950	CalCOFI 1959
.0625	.25	.125	2				
.25	1	.5	4				
1	4	2	6	21			28
4	16	8	7	22	8		28
16	64	32	14	16	15		30
64	256	128	12	31	29	77	23
256	1024	512	13	27	14	20	12
1024	4096	2048	5	17	10	7	5
4096	16384	8192	2	3	2	1	4
		n	65	137	78	105	130
		m	544	569	ca.619	ca.406	410
		s	1466	1317	ca.1389	ca.900	1450
		k	0.136	0.187	0.196	0.207	0.080
		SPAWNING BIOMASS (Millions of Tons)	3.9	2.7	2.7	1.0	0.2

Table 3.11. An example of the sample frequency distribution of Pacific hake eggs and larvae by month (1954-55).

CLASS BOUNDARY		EGGS						LARVAE					
LOWER	UPPER	DEC	JAN	FEB	MAR	APR	TOTAL	DEC	JAN	FEB	MAR	APR	TOTAL
1	4	4	9	2	7	12	34	12	11	3	11	6	43
4	16	11	19	7	22	22	81	11	17	14	24	29	95
16	64	7	14	20	30	25	96	6	22	26	37	27	118
64	256	6	10	18	16	12	62		9	24	22	11	66
256	1024	0	12	12	7	3	34		7	12	7	7	33
1024	4096	1	7	9	6		23		1	9	0		10
4096	16384		4		2		6		1		1		2
16384	65536		2				2						
65536	262144		2				2						
	n	29	79	68	90	74	340	29	68	88	102	80	367
	m	108	4846	406	394	55	1333	10	233	325	157	76	182
	s	376	21169	669	1288	104	10364	12	1019	607	813	141	689
	k	.083	.052	.367	.094	.283	.017	.901	.052	.287	.037	.292	.070

Table 3.12. Worksheet for Kolmogorov-Smirnov test demonstrating the difference between anchovy egg diameters in February and August 1972.

DIAMETER (mm)	AUGUST			FEBRUARY			DIFF. (%)
	FREQUENCY	FREQUENCY (%)	CUMULATIVE FREQUENCY (%)	FREQUENCY	FREQUENCY (%)	CUMULATIVE FREQUENCY (%)	
.5643	3	.5	.5				.5
.5814	12	2.0	2.5				2.5
.5985	30	5.0	7.5				7.5
.6156	75	12.5	20.5				20.0
.6327	87	14.5	34.5				34.5
.6498	96	16.0	50.5	6	1.0	1.0	49.5
.6669	90	15.0	65.5	18	3.0	4.0	61.5
.6840	72	12.0	77.5	84	14.1	18.2	59.3
.7011	51	8.5	86.0	75	12.6	30.8	55.2
.7182	51	8.5	94.5	135	22.7	53.5	41.0
.7353	12	2.0	96.5	96	16.2	69.7	26.8
.7524	6	1.0	97.5	69	11.6	81.3	16.2
.7695	9	1.5	99.0	48	8.1	89.4	9.6
.7866	6	1.0	100.0	30	5.1	94.4	5.6
.8037				24	4.0	98.5	1.5
.8208				6	1.0	99.5	0.5
.8379							0.5
.8550				3	0.5	100.0	0.0
N	600			594			
m	.6614			.7285			
s	.0422			.0368			
$D_{.05}$							5.6%

Table 3.13. Cumulative frequency table for the Kolmogorov-Smirnov test demonstrating the similarity between measurements of a group of anchovy larvae by two people.

LENGTH (mm)	CUMULATIVE FREQUENCY (%)	CUMULATIVE FREQUENCY (%)	DIFFERENCE (%)
2.0	0.5	0.5	0.0
2.5			
3.0	1.6	1.6	0.0
3.5	2.7	5.9	3.2
4.0	14.4	16.2	1.8
4.5	19.8	20.0	0.2
5.0	28.9	35.7	6.8
5.5	36.9	42.2	5.3
6.0	46.0	49.2	3.2
6.5	51.9	53.0	1.1
7.0	59.9	61.6	1.7
7.5	67.4	70.8	3.4
8.0	73.8	76.2	2.4
8.5	78.6	79.5	0.9
9.0	87.7	88.1	0.4
9.5	91.4	90.8	0.6
10.0	94.1	95.1	1.0
10.5	96.8	96.2	0.6
11.0	98.4	97.8	0.6
11.5		98.4	0.0
12.0	98.9	98.9	0.0
12.5	99.5	99.5	0.0
13.0			
13.5			
14.0			
14.5			
15.0			
15.5		100.0	0.5
16.0	100.00		0.0
$D_{.05}$			7.2

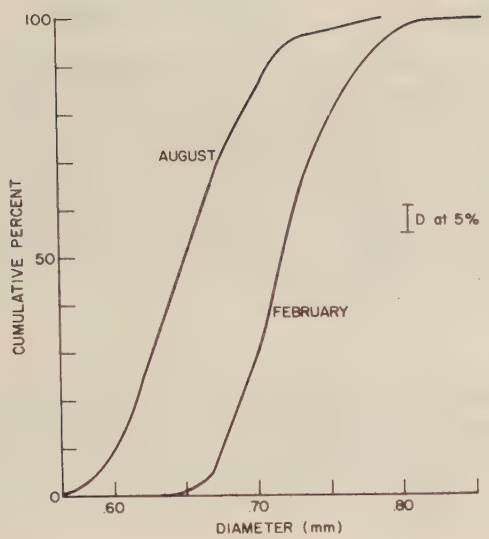


Figure 3.3. Illustration of the Kolmogorov-Smirnov comparison of sample frequency distributions of anchovy egg diameters from samples collected February and August of 1972. The difference (D), that is significant for the 5% level and 594 samples, is shown as a vertical line near the right margin.

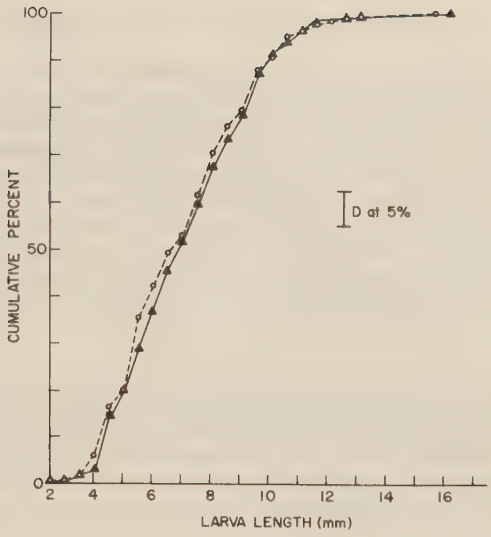


Figure 3.4. Illustration of the Kolmogorov-Smirnov comparison of sample frequency distributions of the lengths of anchovy larvae as measured by two people. The difference (D) that is significant for the 5% level and 356 samples is shown as a vertical line near the right margin.

The same columns are listed for the August samples. The final column is a difference column. For a 5% level of significance, the maximum difference is compared to:

$$D = \frac{136}{\sqrt{N}}$$

$$= \frac{136}{\sqrt{594}} = 5.6\%$$

where "D" is the significant difference level and N is the number of sample measurements (594 in this instance). The fact that the two curves differ by more than 5.6% is taken as conclusive that the difference is significant and that anchovy eggs were smaller in August of 1972 than in February of the same year. The two cumulative percent curves are plotted in Figure 3.3.

In example 2, the question is "do two plankton sorters derive the same length-frequency curve from a sample of 356 anchovy larvae?" In Table 3.13, only the category values and the cumulative frequencies and differences are listed. In this instance, significance at the 5% level is indicated by difference in cumulative percentages greater than 7.2% ($D = 136/\sqrt{356}$). Since no difference exceeding that value occurs, this may be interpreted as supporting the idea that the same 356 larvae were measured essentially the same by two independent plankton sorters. Figure 3.4 illustrates the two cumulative percentage curves for this example. The value "136" in this example is for a large number of samples (>100) to test a difference at the 5% level.

3.1.3. Advice on Data Transformations and Comparisons

It is the recommendation of the senior author that no transformation of data be done without the documented advice of a professional statistician. The widely used log transformation has no explicit or useful method for treating "0" observations. The "0" observations are necessary for determining the edge of the spawning area and are frequent within the spawning area. It is easy to misuse analyses based on log transformations (Bagenal, 1955) and analysis of variance and kindred tests can sense a significant difference in geometric mean when the arithmetic means are identical and detect no difference in geometric means when the arithmetic means are different. In some cases, the log transformation generates a set of values in which the variance is still a function of the mean, thus stabilization of variance is not a guarantee of transformation.

This manual is aimed at standardized conduct and reporting of fish egg and larval surveys, not analysis. In brief, we recommend a generalized distribution which 1) accommodates contagian or patchiness, 2) has explicit zero terms, 3) approximates a poisson at low mean values, and 4) does not assume independence of mean and variance. The negative binomial is our provisional recommendation for data analysis for data of the type collected in the CalCOFI surveys.

3.2. Volumetric Sampling

The earliest objective of quantitative plankton sampling was to take samples from the bottom to the surface under a defined unit of sea surface area. The earliest nets did not accept all the water encountered in the column, and factors for the correction of this bias were calculated by Hensen (1895) and Birge (1895). Reighard (1897) suggested the use of a flowmeter to record volume filtered. Kofoid (1897) pointed out that many smaller organisms were extruded through the meshes. Planktonic organisms were observed to avoid the net (Mackintosh, 1934). The survey procedures recommended in this manual (Section 2) do not eliminate these biases, but with care in equipment design and use, these biases and some of the technical sources of variability can be evaluated or minimized.

3.2.1. Volume and Distribution of Water Filtered

The estimation of spawning biomass requires quantitative samples of the planktonic spawning products expressed as numbers under a unit area of sea surface. This estimation can best be obtained from a tow designed to filter at a constant rate per unit depth from below the deepest occurring egg or larva to the surface. On the continental shelf, and in bays and lakes where quantitative sampling began, the

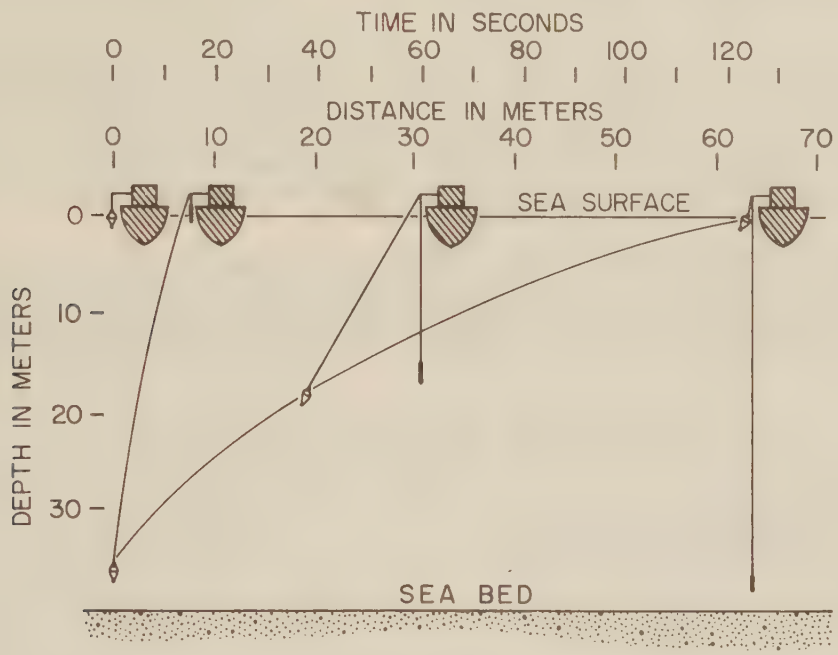


Figure 3.5. An example of the path of a Hensen net when fished vertically with the ship adrift. (From A.C. Simpson, 1959).

vertical tow was the method of choice. This manual recommends an oblique tow instead because the vertical tow cannot be conducted with precision on the high seas.

A.C. Simpson (1959) drew attention to the fact that "vertical" hauls from a drifting ship (Figure 3.5) filtered more water than planned, and he accordingly furnished correction factors for different rates of drifting. Unfortunately, a second bias occurs in stratified waters which cannot be easily corrected. In a vertical tow the assumption is that an evenly integrated tow has been taken from the maximum depth to the surface. It is quite clear that this assumption is systematically violated in vertical tows from drifting ships or from static ships with underlying current stratification. The effect is that the rate of ascent is rapid in the deep portion of the tow, and more and more water is filtered from each unit depth as the surface is approached (Smith, Counts and Clutter, 1968) leading to an unrepresentative sample of the water column biased in favor of the superficial waters.

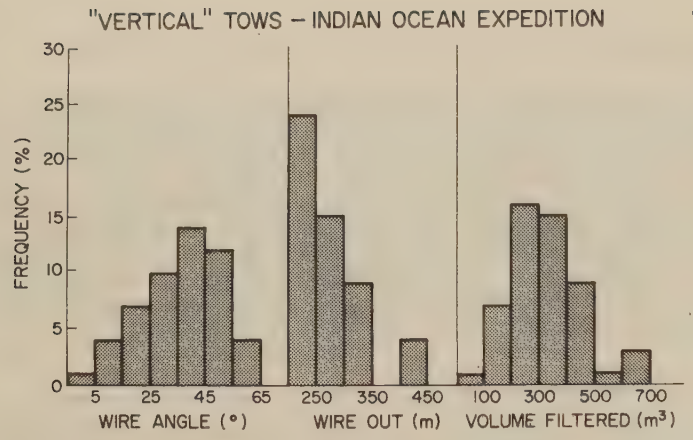


Figure 3.6. A sample of the wire angles, wire out to reach 200 meters depth, and the volume of water filtered during each tow using the Indian Ocean Standard net. (After Motoda, Konno, Kawamura and Osawa, 1963).

This effect will not be serious in well mixed waters.

Swells and the rolling of the ship also cause the vertical tow net to pump portions of its sample back into the sea. The same motions of the ship cause the vertical sample to be accelerated so that portions of the sample lying on the mesh are likely to be extruded through the apertures.

Some examples of the poor quality control of vertical tows are available from recent expeditions. For example, during the Indian Ocean Expedition (Motoda, Konno, Kawamura, and Osawa, 1963), a 1.13 m diameter ($1m^2$ in cross section) net was towed "vertically". Figure 3.6 illustrates the variability in the wire angle, the amount of wire required to reach 200 meters, and the volume of water filtered for these tows. Table 3.14 lists some of the variations in the wire angle of vertical tows made during the Cooperative Study of the Kuroshio (CSK). Here, too, instead of the intended vertical tow, the average tow had a 25° wire angle, the standard deviation (± 2 s.d.) of the average tangent was 0.31, and the actual range of angles was from 9° to 65°. Also, the extended forward disturbances caused by the tow wire augment the usual avoidance problem (Clutter and Anraku, 1968).

Two major faults of the Hensen net are the towing bridle and towing cable ahead of the net (see Section 3.3.2. below) and the inability to locate a stable flow area in which to place a flowmeter (Figure 3.7). The reduction cone causes acceleration of the water near the periphery of the mouth because of the reduced pressure

Table 3.14. Recorded angles of tow for "vertical" tows in the Cooperative Study of the Kuroshio (CSK).

ANGLES OF STRAY	FREQUENCY
0°	16
5	35
10	58
15	52
20	59
25	33
30	59
35	37
40	38
45	11
50	11
55	3
60	0
65	2

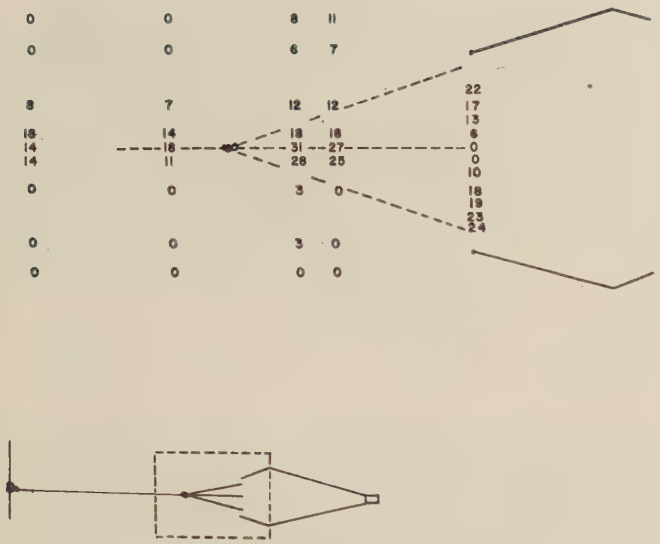


Figure 3.7. A comparison of the accelerations of water in the mouth and several transects ahead of the Hensen net. The numbers represent the difference between the channel flow with and without the net present in cm/sec. The inset illustrates the entire towing arrangement in the test channel.

behind the cone. This effect diminishes toward the center of the net behind the bridle apex. If the towing speed could be made suitably constant, the flow could be calculated, but the degree of acceleration is quite sensitive and dependent on tow speed, therefore the variations experienced in high-seas towing would preclude the collection of precise and accurate filtration performance data.

3.2.1.1. The Oblique Tow

The progress of the oblique tow is actively regulated by the ship when it is underway. In areas where currents are relatively slow, such as the California Current region, it has been found possible to fish plankton nets from the standard 210m depth with reasonable consistency [e.g., 206m ± 16m (2 s.d.)]. In areas with strong undercurrents, such as the Eastern tropical Pacific (Ahlstrom, 1971, 1972) less consistency is achieved (e.g., 203m ± 37m) although the variation is still within acceptable limits. One important factor to consider when comparing oblique tows with vertical tows is that the greatest errors in the oblique tows occur at depth and the error diminishes toward the surface (Figure 3.8). The vertical tow path error is cumulative toward the surface. Variation in the volume filtered in a series of oblique tows is usually much less (e.g., volume filtered = $649m^3 \pm 86m^3$ for 165 tows of the CalCOFI net) than it is in vertical tows (e.g., the Indian Ocean Standard net, Fig. 3.6).

The tow-by-tow variation in these properties can be measured adequately from the surface by monitoring the wire angle closely. Fig. 3.9 demonstrates this by showing the similarity of tow paths for an ideal tow as determined from wire angle readings and a bathykymograph. About the best that can actually be accomplished in maintaining a straight oblique tow path on a net tow under ideal conditions is illustrated in Figure 3.10. Part "a" of that figure shows the ideal tow path, the tow path estimated by a bathykymograph (part "b"), and the time course of the rate of ascent. In the ideal standard tow, 20 meters of wire are retrieved per minute with a wire angle of 45°. The ideal rate of ascent is 14 meters per minute. In the tow in question (Fig. 3.10), the rate of ascent varied ± 30% from the ideal rate. An example of the maximum acceptable variation from the ideal tow path, for the purposes of standardization, is illustrated in Figure 3.11. In this example the rate of ascent varied ± 84% from the ideal. Where the rate of ascent is higher than average, less water is filtered per unit depth. The variation in tow path can be evaluated by converting the wire angle readings, which are made at 30 second intervals during the tow, to tangents and calculating the average tangent and standard deviation for the tow (Table 3.15). Standards can be established ahead of time for accepting or repeating tows. If the standard deviation of the average tangent is too great (e.g., 2 s.d. $\geq$ 0.1) the tow should probably be repeated. One way to assure a good tow is to maintain the wire between 42° and 48° (45° ± 3°). The constancy that is possible is exemplified by a series of 204 high-seas tows made in a temperate area. The average tangent was 1.077 (± 2 s.d. = 0.084) with 95% fiducial limits of 0.91 - 1.24.

The importance of the variation in the rate of ascent is underscored by the stratified nature of the oceanic environment. One of the earlier explanations for "patchiness" was the allegation of uneven ascent of the net through stratified layers of sardine eggs (Sette and Ahlstrom, 1948). This feature was also used to explain the fact that the use of volume filtration data was not significant in the

analysis of variance of egg counts. The uneven passage of the net through the water column should be carefully avoided. Further effects of this uneven rate of ascent include the increased extrusion of flexible organisms during the faster part of this retrieval, and the possibility of the flowmeter stopping during the slower part of the fluctuation in rate of ascent.

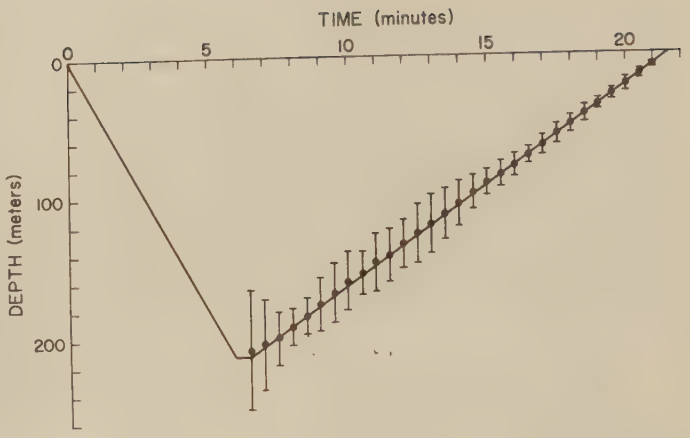


Figure 3.8. The ideal tow path in the CalCOFI oblique tow to 210 meters (solid line) and the average depth at each 10 meters recovered ± 2 standard deviations in a test series of 14 tows.

3.2.1.2. Clogging

At times, material in the seawater may clog the mesh of the net thus lowering the filtering capacity of the netting. As long as the flowmeter continues to function, this deficit in water filtered will be monitored. As can be seen from Fig. 3.13 however, the sample taken gets progressively less representative with time. The tendency is to undersample the surface layer if clogging proceeds throughout the tow. In Figure 3.12 the trail of dye streaks through the netting indicates that clogging occurs evenly over the entire net surface, not just near the cod end. The rapidity with which clogging can occur is illustrated in Fig. 3.13. If clogging is routinely encountered, the only solution is to increase the filtering area (Smith, Counts and Clutter, 1968; Tranter and Smith, 1968) by adding extra panels of mesh in the cylindrical part of the net.

3.2.2. Avoidance

The use of ichthyoplankton surveys to monitor spawning biomass requires constancy of larval size distribution in the samples obtained. The size distribution of larvae captured in plankton nets is affected by two factors. In particular, as larvae grow they appear to undergo heavy mortality while at the same time they improve their ability to evade plankton nets. Understanding the relative importance of mortality and avoidance in the declining catch of fish larvae with increase in size should aid in the interpretation and understanding of the role of larvae mortality and recruitment of future stocks.

Research on avoidance was extensively reviewed by Clutter and Anraku (1968). Further work on an "ultimate" sampler by Murphy and Clutter (1972) points out the extreme nature of larval avoidance (Figure 3.14) and further explains why the search for an "ideal" sampler has not been very productive. It appears likely that the day-night difference in avoidance is only a small part of the total avoidance problem, the bulk of the avoidance being performed as well in the dark as in the light. Barkley (1964; 1972) described the relative capacities of larvae to avoid when given advance warning of the approach of the sampler. He indicated that the response starts three to four mouth diameters ahead of the net. Larger and faster tow nets may alter the ability of larvae to avoid very little because they intensify the disturbance and project it further ahead of the net.

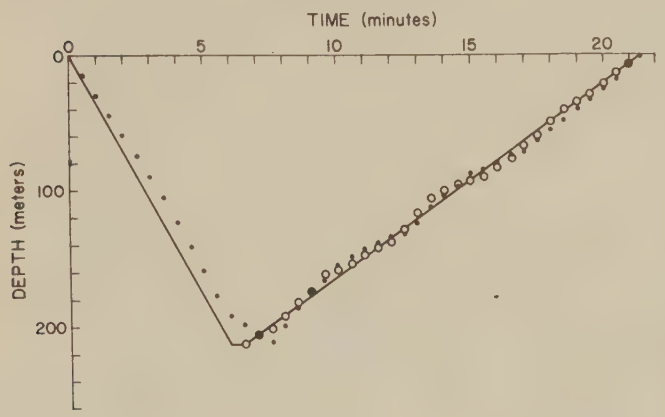
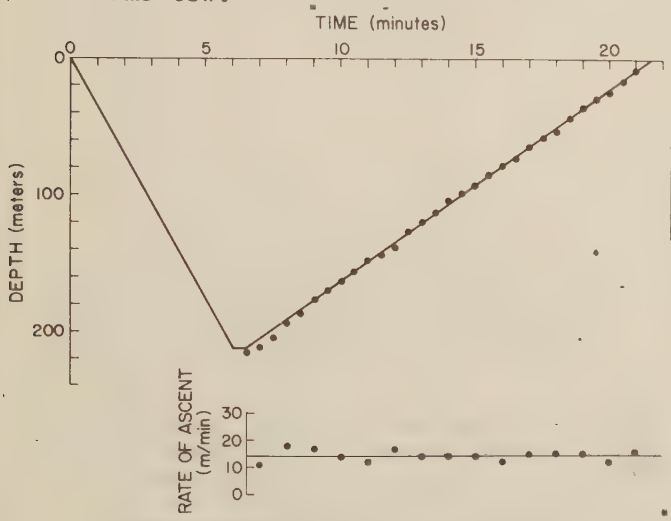
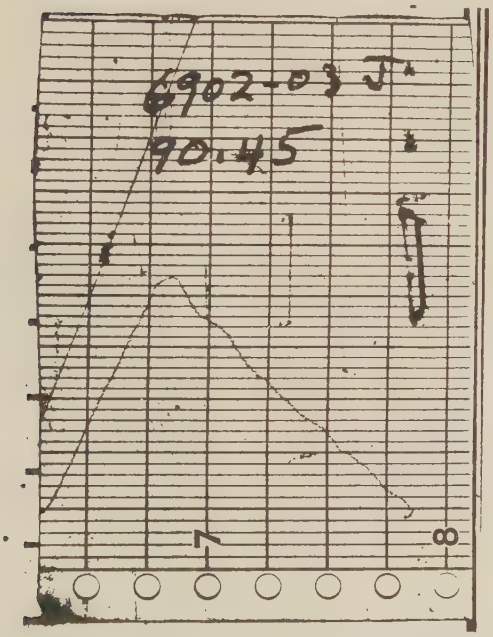
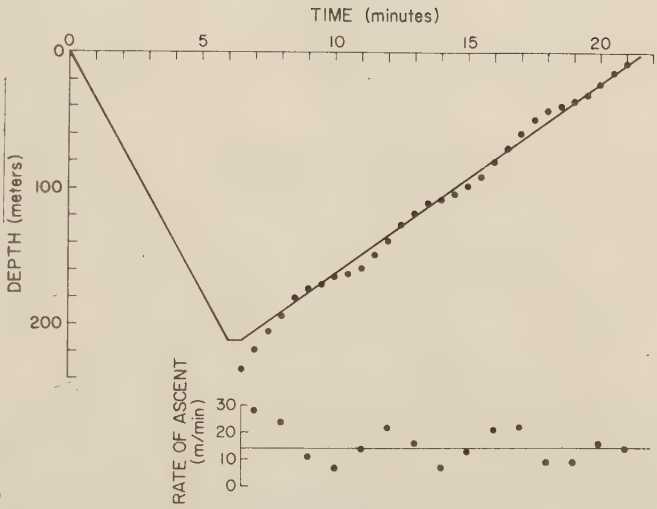


Figure 3.9. The ideal tow path of the CalCOFI net (solid line), the depth measured by bathykymograph (solid dots) and the depth estimated by the angle of stray and the wire out (open circles).

Figure 3.10. An ideal tow. a) In the upper figure the ideal tow path is illustrated (solid line) and the attained depths, determined by a bathykymograph, are solid dots. The first difference in the meter's depth each minute is illustrated in the lower portion of the drawing. This is the critical measure of the volume of water filtered for each unit depth. b) A bathykymograph trace of the same tow.







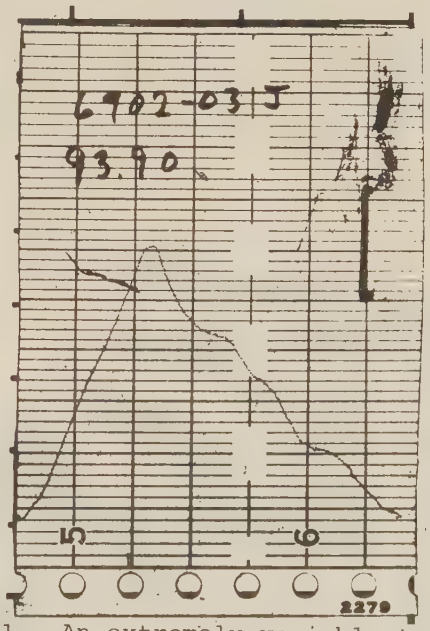


Figure 3.11. An extremely variable tow. a) In the upper figure the ideal tow path is illustrated (solid line) and the attained depths, determined by a bathykymograph, are solid dots. The first difference in the meter's depth each minute is illustrated in the lower portion of the drawing. This is the critical measure of the volume of water filtered for each unit depth. b) A bathykymograph trace of the same tow.

Table 3.15. An estimation of the variation in towing path derived from the average tangent and standard deviation of the tangents.

θ	tan θ	f
50°	1.1918	1
49	1.1504	2
48	1.1106	8
47	1.0724	8
46	1.0355	7
45	1.0000	4
$\bar{T}$	1.0735 (=47°)	
S_T	0.0486	
95% Fiducial Limits	.9782 - 1.1688 (= 44°-49°)	

Some mechanical cues for avoidance are available both day and night. Smith and Clutter (1965), Mahnken and Jossi (1967), and Tranter and Smith (1968) described the accelerations of water in front of the plankton net caused by the legs of the towing bridle, the towing bridle apex, and the towing cable. A simulation of a thoroughly clogged net (Fig. 3.15) illustrates the fact that the advanced warning due to a "bow-wave" effect is probably limited to the region of approximately one mouth diameter ahead of the net. The bridles without a net propagate measurable disturbances (Fig. 3.16) along the towing line as far as it proceeds ahead of the net, and particularly strong disturbances occur at the bridle apex. This is an important consideration in vertically towed nets. The absence of these disturbances is one of the most important features of the recommended Bongo nets.

The main reason for understanding day-night differences is to be able to correct sample data for size and time-of-day in order to make all the data obtained comparable for use in estimating spawning biomass. Lenarz (1973) found that the decreased catch per unit larval length for a set of CalCOFI tows increased in the order sardine, anchovy, hake and jack mackerel. To transform the data to a comparable basis regardless of day or night, or larval size, he fitted the equation:

$$\ln (N_{sd}/N_{s_o d}) = \ln A - B (s - s_o) + C_d (s - s_o) + \ln (E_{sd})$$

"N_{sd}" is the number of larvae of size "s" caught during time period "d", and "s" takes values s_o s_1, $s_2 \ldots s_t$ where "s_o" is the modal size group and "s_t" is the largest size larvae with significant catch; "d" is the time of day, i.e., day (-1), or night (0)*, A is a constant, B is

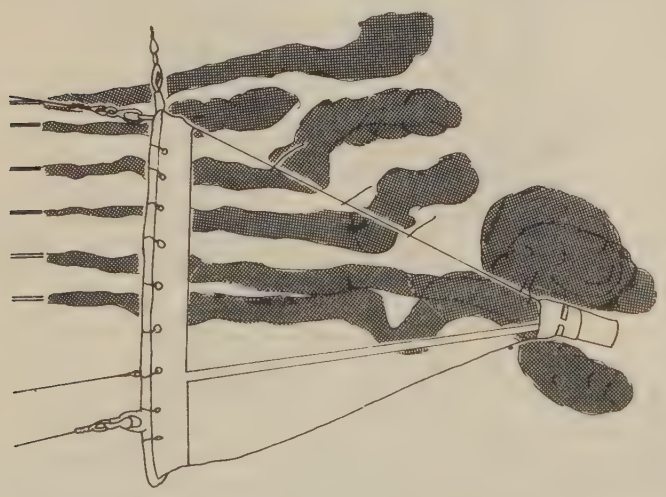


Figure 3.12. A composite drawing made from photographs of dye passing through an experimental net in a towing chamber. The yarn tabs perpendicular to the filtering surface are a separate indicator of flow.

*This variable was incorrectly identified as day (0), night (1) by Lenarz (1973).

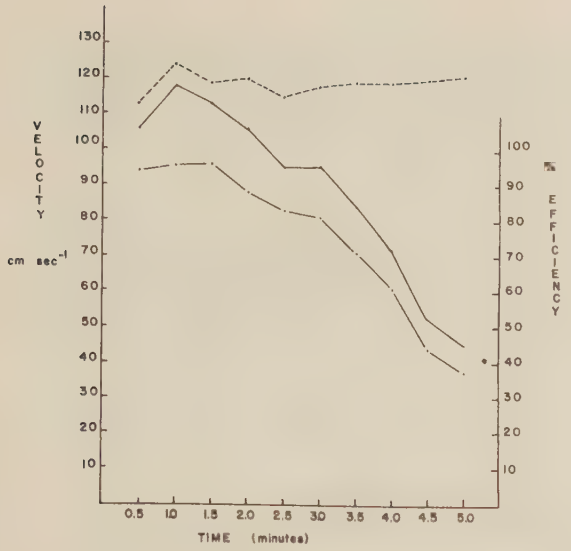


Figure 3.13. The clogging of a WP-2 (working party 2) 0.202 mm mesh conical net (UNESCO, 1968) under tow in Southern California nearshore water.

----- towing speed indicated by a telemetering flowmeter outside the net.

_____ rate of flow through the mouth of the net indicated by a telemetering flowmeter inside the mouth of the net.

. ___ . ratio of the inside and outside readings times 100 (% efficiency)

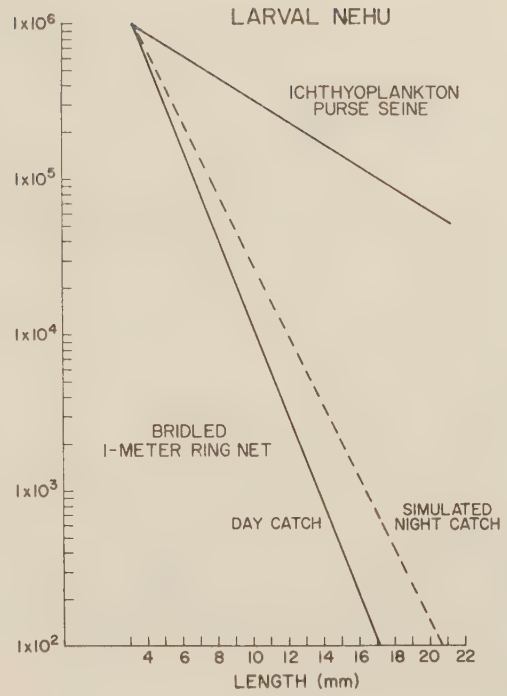


Figure 3.14. An example of the degree of avoidance of larvae (in this case larval nehu, Stolephorus purpureus) by day from a bridled ring net as compared to a plankton purse seine. (From Murphy and Clutter, 1972)

the instantaneous rate of decline in catch with size, "C" is the instantaneous rate of increase of the night to day ratio with size, and "E_{sd}" is the error term assumed to be independent of "s" and "d" and to have a log normal distribution.

The solutions to this equation were:

Species	B	C
Sardine	0.22	0.20
Anchovy	0.42	0.16
Hake	0.48	0.21
Jack Mackerel	1.18	-0.11

If the negative value of "C" for jack mackerel were significant it would mean that there is an increased tendency to be caught at a higher rate during daylight than during night. The smallness of the coefficient probably does not warrant such a conclusion and an alternate explanation is that jack mackerel of all sizes avoid the net as well at night as during the day.

3.2.3. Mesh Retention

The retention of small larvae is as important to the effective calculation of spawning biomass as the capture of larger larvae and juveniles is to prediction of recruitment. Probably more than half the anchovy larvae in the ocean, for example, are less than 4.25 mm long and extremely vulnerable to changes in filtration

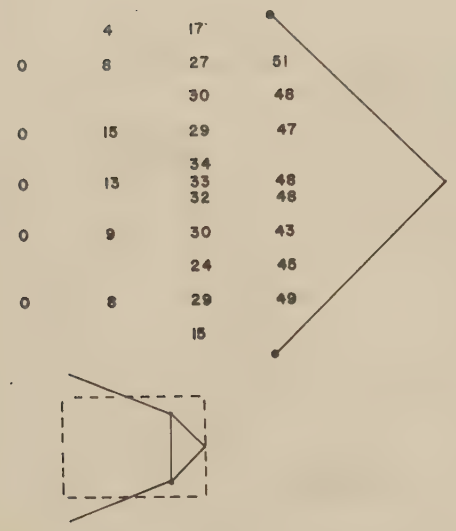


Figure 3.15. A one meter diameter solid cone suspended in a towing channel. The transects of figures represent the difference in channel velocity at those locations with and without the net and are expressed in cm/sec. (From Tranter and Smith, 1968).

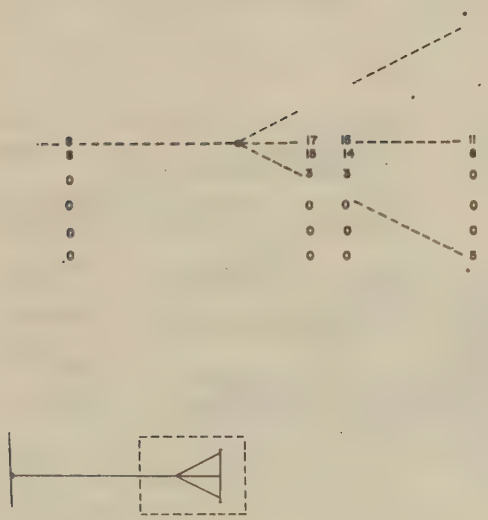


Figure 3.16. An illustration of the accelerations ahead of the towing apparatus of a net when no net is present. (From Tranter and Smith, 1972).

pressure and escapement and extrusion through the meshes. Lenarz (1972) estimated the retention ratio of anchovy and sardine in nets towed at constant speed and found the relationships:

$$s = a + bL$$

Where s is the mesh retention ratio of larvae of size "L" (less than 5.75 mm SL), L is the length of larvae, "a" is the usual intercept of a linear regression, and "b" is the slope.

For the silk CalCOFI net, the relationships were:

	a	b
Sardine	-.2382	0.2107
Anchovy	-.1942	0.1945

and the retention ratio by length was:

Length (mm SL)	Anchovy	Sardine
2.50	0.292	0.289
3.75	0.535	0.552
4.75	0.730	0.763
5.75	0.924	0.973

The static and dynamic relationships of mesh retention can be discussed. In Fig. 3.17 the width and diagonal measurements of four different mesh sizes are compared for their retention capabilities of anchovy and hake larvae. It can be seen that larvae are captured because of their length but they are retained because of their body depth which becomes a critical dimension. Another factor to consider is the variability in mesh size within the netting. For example this variability is greater in silk mesh nets than in those made of nylon (Fig. 3.18). Mesh variability is reviewed by Heron (1968). A good review of factors involved with mesh selection is given by Vannucci (1968).

In nets with high filtering rates, the complexities of extrusion and larval damage are encountered. These rates are functions of the square of the velocity and the ratio of mesh surface area to mouth diameter (Tranter and Smith, 1968). As yet, no generalizations can be made on the relationship between larval size, mesh size, towing speed and variance in towing speed. Until this analysis is completed, low constant towing speeds (1.5-2.0 knots) should be maintained. This is the primary consideration behind the recommendation of the "slow" Bongo nets.

An example of the severity of extrusion with increased towing speed can be seen in these preliminary data on percent

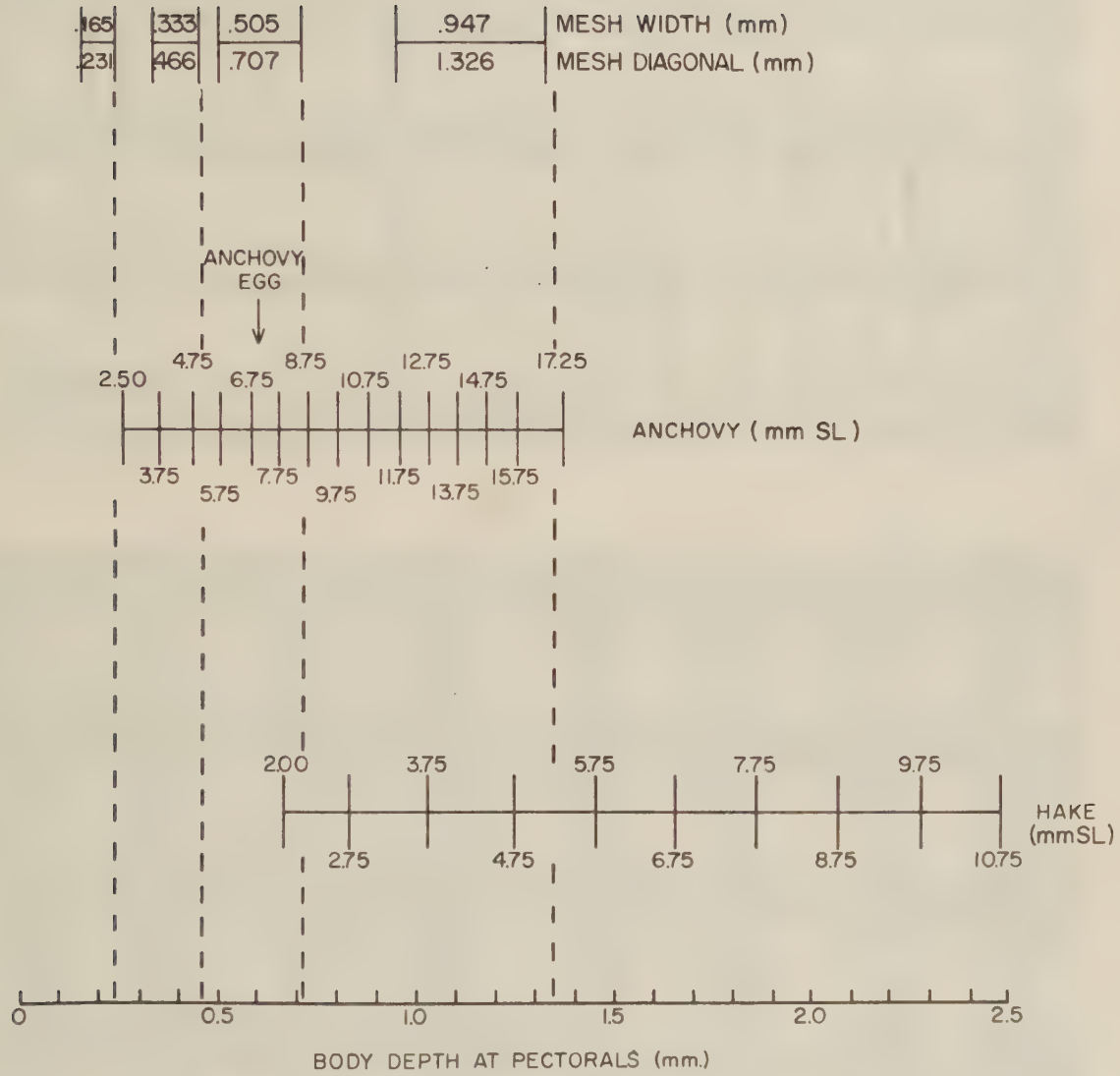


Figure 3.17. An example of retention characteristics of various mesh sizes. The mesh width (left bar on upper line) and diagonal (right bar on upper line) are compared with standard length and corresponding body depth of anchovy and hake. The dashed line corresponds to the level of absolute retention. (Modified from Smith, 1972*)

*Smith, P.E. 1972. Extrusion. MARMAP, Technical Memorandum #6, June 7, 1972. Unpublished manuscript.

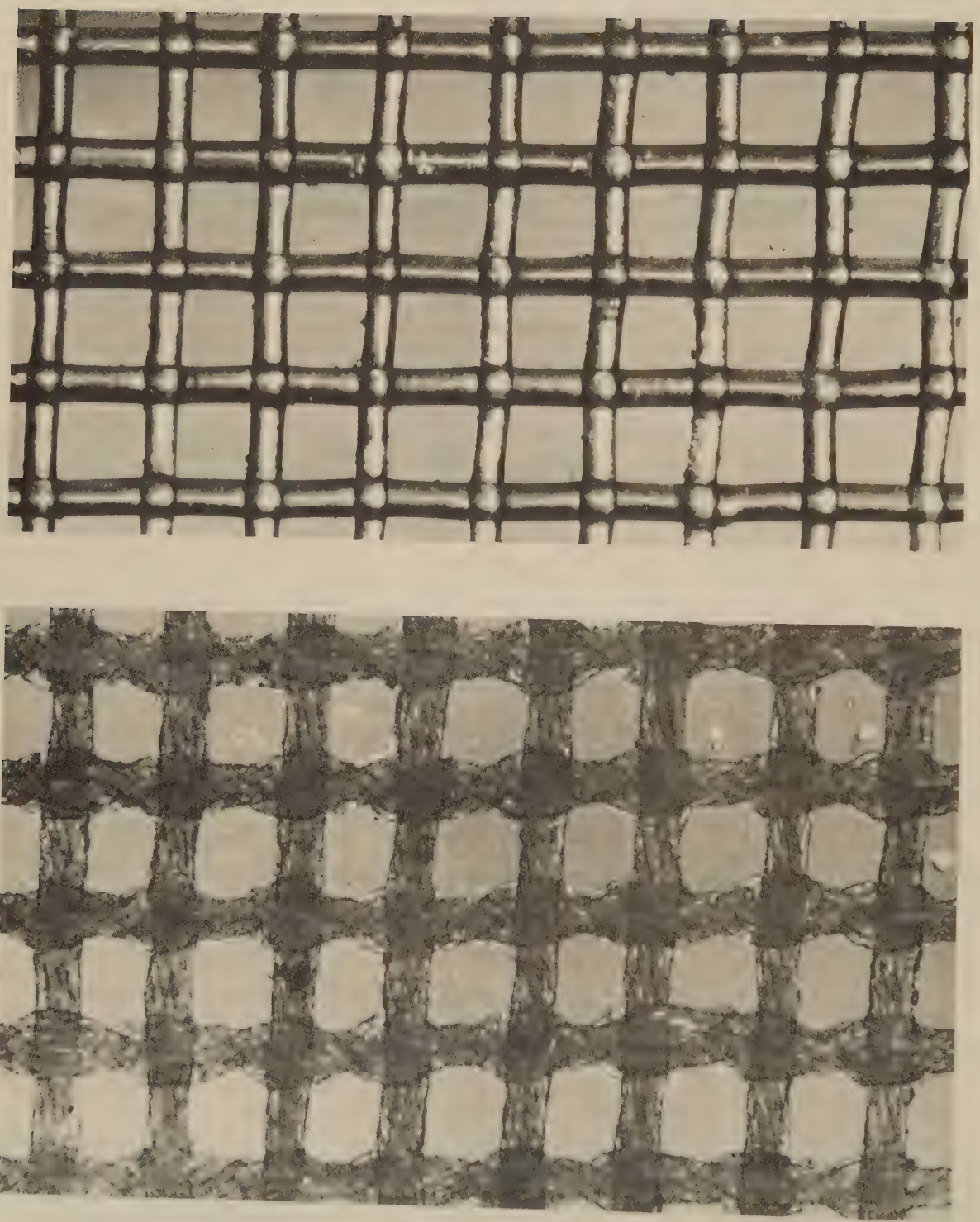


Figure 3.18. Above, 0.505 mm monofilament mesh (.505 Nitex*). Below, 0.55 mm mesh silk mesh [30xxx (extra heavy) GG (grit gauze)].

of anchovy larvae retained from Smith (1972)* [based on ideas set forth by Saville (1959) and Heron (1968)]:

Anchovy Standard Length (mm)	Towing Speed (Knots)			
	1.5 **	2.00	3.15	4.14
2.50	.292	.478	.154	.063
3.75	.535	.565	.474	.137
4.75	.730	.777	.807	.285
5.75	.924	1	.802	.329
6.75	1	1	.752	.423
7.75	1	1	1	.826

3.2.4. Uneven Tow Trajectories

Current towing methods do not permit an analysis of the assumption that equal volumes of water are filtered at each depth. While these differences can be measured, and the effect on a given vertical distribution estimated, each tow in a survey must either be accepted or rejected, for no "standardization" or "adjustment" is tractable. Until directed research is completed on this problem, one can only say that a potential problem exists and that its magnitude is less than the variance associated with the normal level of patchiness encountered.

* Smith, P.E. 1972, Extrusion, MARMAP Technical Memorandum #3, May 22, 1972. Unpublished Manuscript.
Smith, P.E. 1972, Extrusion, MARMAP Technical Memorandum #6, June 7, 1972. Unpublished Manuscript.

**CalCOFI silk/0.55 mm mesh (Lenarz, 1972); all others Bongo Nitex/0.505 mm mesh.

3.3. Sampling to Determine Spawning Area and Time

The geographic, vertical, and seasonal boundaries of spawning must be well defined for the analysis of spawning biomass. Since the primary costs of ichthyoplankton surveys are related to distance traveled by ship, understanding these boundaries may permit substantial reduction of sampling effort between the exploratory phase and the monitoring phase of the survey program. Also, the spawning products diffuse with time and are transported off the spawning grounds, and migrations of adults may occur during spawning. Both of these factors may affect the seasonal placement of boundaries.

3.3.1. Geographic Distribution

The pelagic habitat appears to permit spawning over vast areas. For example, the northern anchovy of the California Current regularly spawns over an area of 50 to 100 thousand square miles. The Pacific saury spawns so widely, beyond the usual boundaries of the CalCOFI survey grid, that a satisfactory westerly boundary has never been usefully defined (Figure 3.19) and it is possible that the distribution is trans-Pacific. In this figure, the incidence of saury occurrence has been plotted at stations sporadically occupied around the periphery of the usual CalCOFI area. Were it to become necessary, these peripheral stations could be used to design a survey to surround the spawning area of this species. The basic ability to define the geographic boundaries of spawning is one criterion for deciding whether the ichthyoplankton survey technique is feasible for a particular fish species.

Figure 3.20 shows the spawning of jack mackerel (Trachurus symmetricus) was undoubtedly occurring to an important extent outside the usually surveyed area but the north-south and the seasonal distributions appeared to be adequately enclosed. In some instances such as these, a conservative estimate of spawning biomass may be made (Ahlstrom, 1968; Gulland, 1970) and plans may be modified to enclose the spawning in subsequent surveys. In this way the monitoring surveys for sardine and anchovy may be used as the exploratory surveys of other species with only small technical costs added to the major goal of the survey.

Colebrook (1973) has examined alternative strategies in sampling simulated seasonal and geographic patterns. His model demonstrated that under the conditions simulated there is little difference among random, stratified random, grid, and route transects over a region at several degrees of pattern intensity. However, the grid was clearly more expensive than the others in terms of the travel distance to occupy stations. Colebrook (1973) also emphasized that the evenly spaced grid in time and space has advantages for mutifactorial treatment of data. It should also be kept in mind that many oceanographic techniques require a station transect normal to the coast, and great economies are available for combined fisheries, biological and oceanographic surveys, since ship-time is the greatest unit cost of each type of survey.

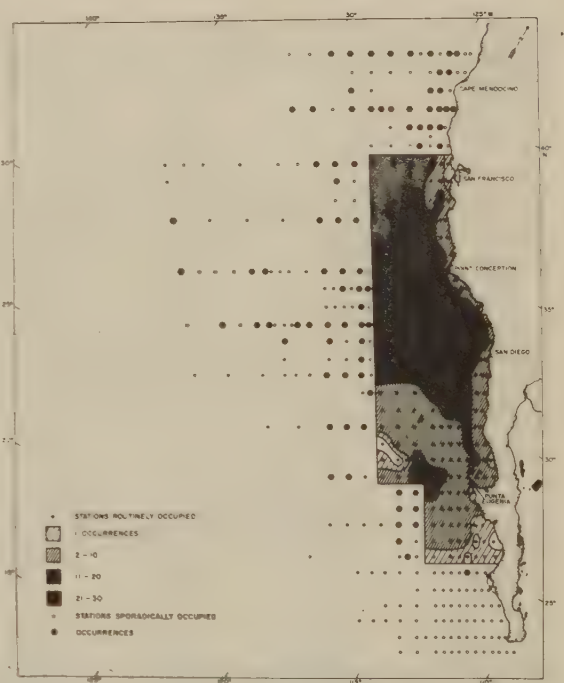


Figure 3.19. A composite drawing showing the incidence of saury eggs in the usual CalCOFI survey area and around the periphery. (From Smith, Ahlstrom, and Casey, 1970)

Switzer (1967) has examined the loss of precision resulting from the change from an even grid to a grid which concentrates stations along lines and separates the lines by an equivalent proportion. For example, the error level from a square grid increases only 10% when a 2:1 rectangular grid is used and

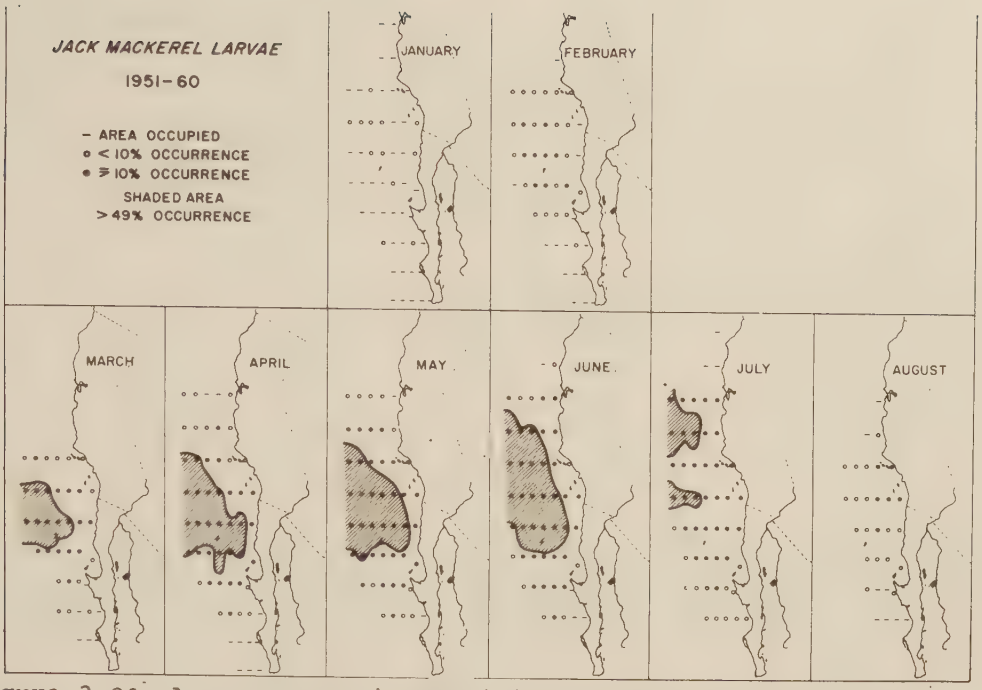


Figure 3.20. Average spawning position for the jack mackerel, Trachurus symmetricus, over a decade. (After Kramer and Smith, 1970)

the distance traveled is reduced 30%. It is recommended that a pilot-study survey with square grid be occupied and the alternative results from increasing rectangularity be analyzed for the optimum grid pattern.

3.3.2. Vertical Distribution

The determination of the extent of vertical distribution of a particular species is simplified by the observation that most pelagic spawning by fish occurs in the upper few hundred meters (Ahlstrom, 1959). Proper survey design is necessary to assure that the vertical distribution of spawning products and the subsequent movements of the eggs and larvae in the vertical plane will be delimited or described.

There are ecological and physiological aspects of vertical distribution. The ecological features, which involve the vertical coincidence of the fish larvae and their common food, competitors, and predators, are outside the scope of this manual. A physiological feature that is important in estimating spawning biomass is the temperature at which development is taking place. This information is necessary to calculate the residence time and abundance of the larvae. Thus, in ichthyoplankton surveys for spawning biomass estimates, the sample is taken from below the deepest larva (or at least through a consistent fraction of the larvae), and the larval abundance is reported as the number under a unit sea surface area (e.g., 10 square meters). This contrasts with ecological studies which report larva abundance as numbers per unit volume at given depths and which also require more precise temperature corrections for residence time.

A method for determining the proportions of larvae of a given species is illustrated in Table 3.16 and Figure 3.21 for anchovy and hake larvae collected in horizontally towed opening and closing Leavitt nets (Ahlstrom, 1959). Basically, the depth range is divided among the horizontal hauls by assuming that the mid-depth of each tow is representative for half the distance to the adjacent mid-depth above and below. At the surface, the upper tow represents depths all the way to the

Table 3.16. A method for estimating the depth distribution of anchovy and hake larvae.

REPRESENTED DEPTH (m)	DEPTH RANGE (m)	MID-DEPTH (m)	ANCHOVY				HAKE			
			$N/1000m^3$	$N/10m^2$	%	CUM. %	$N/1000m^3$	$N/10m^2$	%	CUM. %
0 -5	5	2	234	11.70	11.8	11.8	14	.70	0.7	0.7
5 -13.5	8.5	8	253	21.51	21.7	33.5	16	1.36	1.3	2.0
13.5-23.5	10	19	136	13.60	13.7	47.2	5	.50	.5	2.5
23.5-34.5	11	28	100	11.00	11.1	58.4	3	.33	.3	2.8
34.5-48.5	14	41	155	21.00	21.2	79.5	12	1.68	1.7	4.5
48.5-64	15.5	56	99	15.35	15.5	95.0	57	8.84	8.7	13.2
64 -80	16	72	19	3.04	3.1	98.1	346	55.36	54.4	67.6
80 -96.5	16.5	88	11	1.82	1.8	99.9	44	7.26	7.1	74.7
96.5-121.5	25	105	0.2	.05	.1	100.0	17	4.25	4.2	78.9
121.5-176.5	55	138	0	0	0		27	14.85	14.6	93.5
176.5-250	73.5	215	0				9	6.62	6.5	100.0
250 -320	70	285	0				0			
TOTAL TO 320 METERS				99.07				101.75		

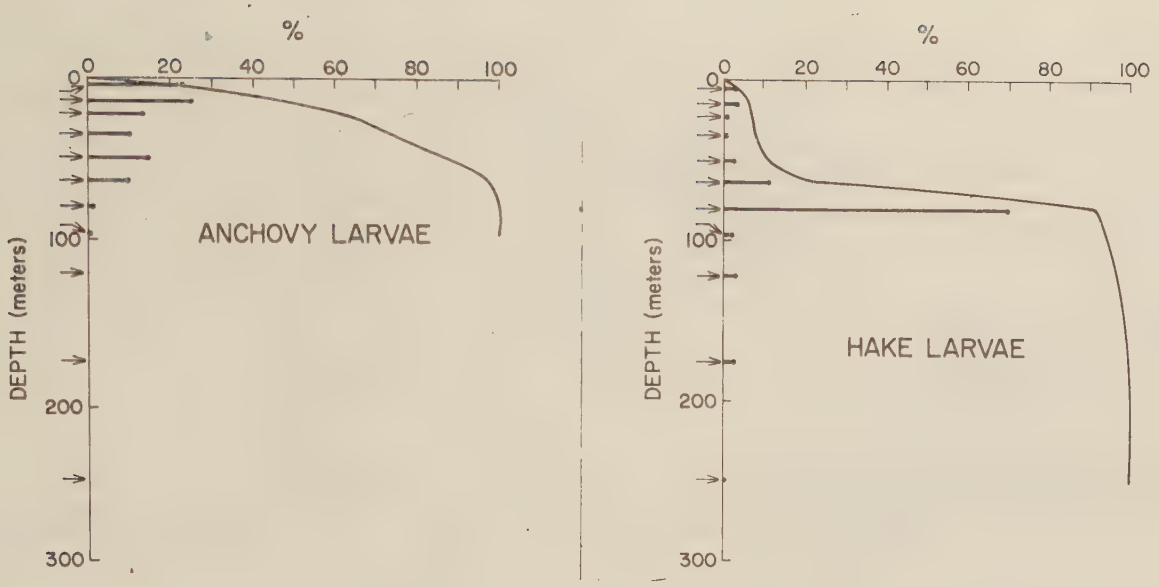


Figure 3.21. The depth distributions of anchovy larvae and hake larvae. The solid line is the cumulative percent curve and the individual bars at the indicated depths are the percent values of incidence. The arrows indicate the horizontal sampling levels. (From Ahlstrom, 1959)

surface . The lowest tow is assumed to represent as much depth below the mid-depth as above. The number of larvae per $1000m^3$ at each depth is transformed to the number of larvae per $10m^2$ sea surface area between the limits represented by the tow by the following equation:

$$C = C_V \left(\frac{SR}{V} \right)$$

Where C is the number per unit sea surface area; C_V is the number per unit of volume (234 anchovy larvae per $1000m^3$); S is the unit sea surface ($10\ m^2$ in this example); R is the range of depth for the sample (5 m in this example); V is the unit volume ($1000\ m^3$ in this example). Thus anchovy between the surface and 5 m:

$$C = 234 \left(\frac{10 \times 5}{1000} \right) = 11.70$$

anchovy larvae per $10\ m^2$ from 0 - 5 meters depth. In this instance, the depth intervals are uneven. For some purposes, it is more convenient to use evenly spaced intervals. It now seems likely that stratification is more pronounced than earlier thought (Longhurst, 1967), which implies that much finer depth intervals than the above example will be necessary to increase the precision of depth distribution analysis. It is likely that the above type of analysis will suffice for determining the maximum depth of tow for species in the upper 300 meters.

3.3.3. Seasonal Distribution

Seasonal distribution of sampling will depend on the characteristics of the spawning season by species and the residence time of the spawning products in the sea. For example, sampling in polar areas can be expected to center around a brief spawning period with an extended residence time of planktonic stages. In tropical areas the sharp seasonal cycle may be absent and the residence time of the spawning products may be quite short. In temperate areas these characteristics are, of course, intermediate, with the added complexity that polar or tropical waters may actually be transported into the temperate areas to varying degrees. If a fishery on the target species exists in an area, gonad samples of the catch may be the best index of when ichthyoplankton surveys should be conducted. In the CalCOFI area, the four months January, April, July and October have been most routinely occupied with supplemental cruises made during the height (February, March, May and June) of

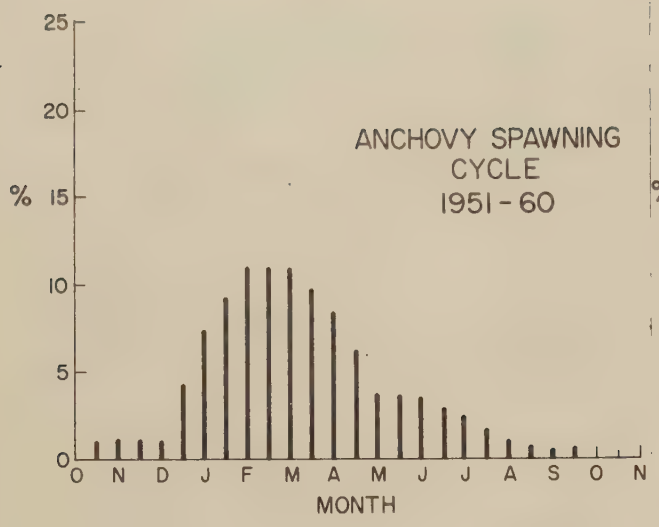


Figure 3.22. The spawning cycle of the northern anchovy, Engraulis mordax, in the California Current region.

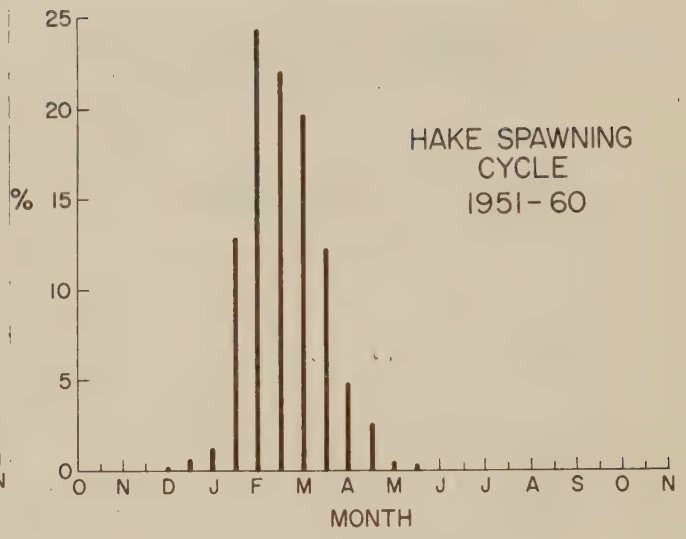


Figure 3.23. The spawning cycle of the hake, Merluccius productus, in the California Current region.

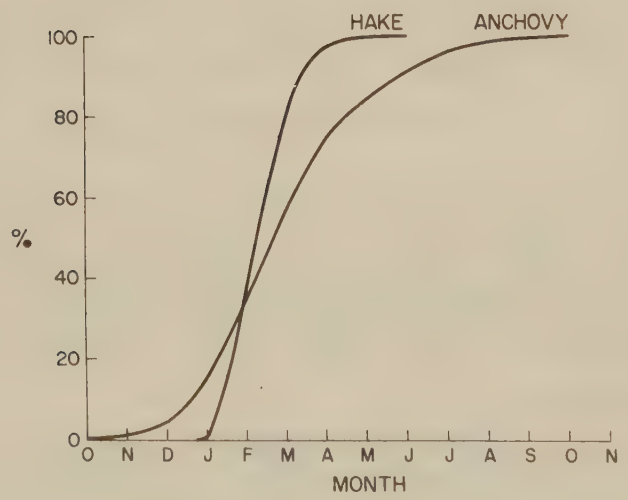


Figure 3.24. The cumulative percentage catch cycles of the larvae of hake and anchovy in the California Current region.

the spawning season of most species.

As in the case of geographic distribution, the purpose of monitoring is not to describe in detail the seasonal distribution of spawning but to get a representative sample of the spawning. For example, a monthly larval index for anchovy varies from a value of 5 to 112 with an average of 44. The larval index for four quarterly cruises is 48 for January, April, July and October, 42 for February, May, August and November, and 40 for March, June, September and December. The range of estimates is only 20% of the mean value for four cruises. For alternate monthly cruises, the larval index is 44 for the set of six cruises beginning in January and 43 for the six cruises beginning in February. The average anchovy spawning cycle is illustrated in Figure 3.22. Thus, four cruises conducted at three-month intervals would probably be adequate to sample anchovy, or similarly spawning species which have extended periods, for spawning biomass estimates. However, six cruises, two each quarter in the spawning season and one each quarter out of the normal spawning season, would assure a more precise estimate of spawning biomass.

The same monthly larval index for hake averages 28 and the three quarterly cruise possibilities are 20, 42, and 33. The two alternate month cruise series yield larval indices of 24 and 33. Thus, to adequately sample hake, or similarly spawaning species which have one or more brief spawning periods, requires more frequent and concentrated cruises. The hake spawning cycle off California is illustrated in Figure 3.23 and the cumulative percent frequency curve for anchovy and hake (beginning in October, the seasonal minimum for spawning) is shown in Figure 3.24.

For some species, the seasonal and geographic distributions of spawning interact and cause some sampling complexity. These cases are typified by the spawning that begins at one end of the range of the adult and spreads across the entire range by the end of the spawning season. The statistical difficulty in this case is that the survey cruise pattern may at times be conducted in the same direction that the spawning is spreading, thus apparently extending the spawning season by region. Conversely, the cruise may be conducted so that the stations are taken across the

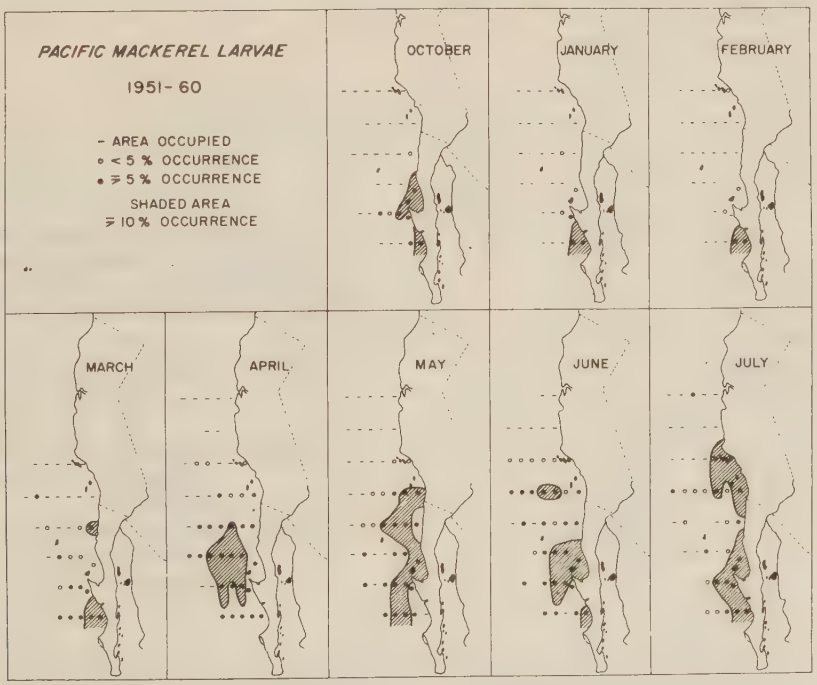


Figure 3.25. The spawning regions and seasons of Pacific mackerel, Scomber japonicus, in the most completely sampled months of the CalCOFI surveys. (From Kramer and Smith, 1970c).

area against the trend of spawning appearing to give a shortened season by region.

An example of incomplete seasonal coverage is shown in Figure 3.25. In this case, the Pacific mackerel (Scomber japonicus) is shown to be spawning in significant amounts in the region to the south of the survey area. It would be assumed that spawning was also occurring in the missing months of August, September, November and December. It is possible that the months of August or September could contain the peak spawning. Here, as in the jack mackerel example (Figure 3.20) the calculated spawning biomass is assumed to be minimal. These data have been collected incidental to ichthyoplankton surveys designed for the Pacific sardine and may be used to modify future surveys to enclose the Pacific mackerel spawning areas and times.

3.3.4. Oceanic Transport and Diffusion

Monitoring spawning biomass by ichthyoplankton surveys will normally include the sampling of several days' spawning products (Sections 3.4, 3.5). These data may be pooled to obtain an egg or larva regional census estimate (Section 2.4). It may also be important to estimate the transport of eggs or larvae into and out of the region. For example, regions that are transported from will exhibit an unnaturally high apparent mortality rate because older larvae have more chance of being transported away. This causes over-sampling in the "receiving" area. Figure 3.26 shows the characteristic lowering of the incidence of young larvae and elevation of the relative abundance of older larvae due to increased chances of being transported offshore.

The general problem of diffusion was treated by Hirano and Fujimoto (1968) and some preliminary calculations of the effect of diffusion on the statistical distribution of sardine egg samples have been made (Smith, 1973). Smith concluded that sardine eggs are spawned in a mosaic pattern of fish school dimensions, and the eggs at the perimeter subsequently disperse to a condition of randomness at the scale of the plankton sample. While an "average" condition leads to the dispersal of egg and larva patches with time, there is little information yet on the variability

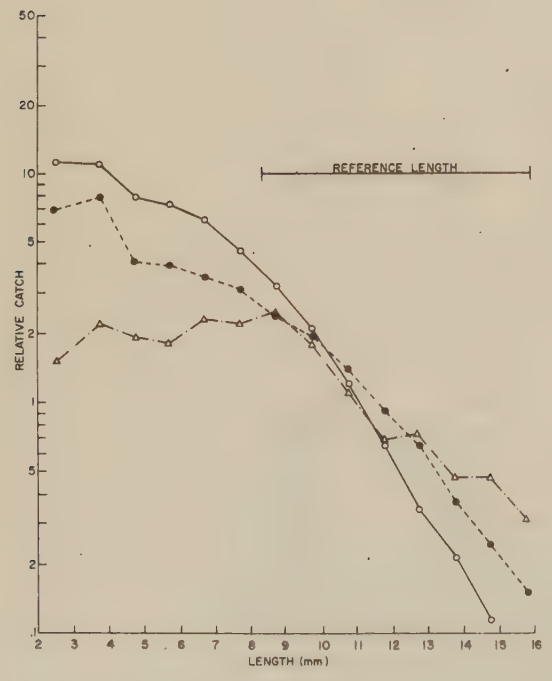


Figure 3.26. An example of a larva catch curve which has been influenced by transport of larvae away from the coast. (From Smith, 1972)

o——o catch curve of anchovy larvae near the coast

●---● catch curve of anchovy larvae 80 miles off the coast

Δ-.-Δ catch curve of anchovy larvae 160 miles off the coast

of this rate. Slight variations in this rate could measurably alter the chances of getting or missing the occasional large samples which characterize the sampling of pelagic spawning products. Until this property is understood, it would be useful to measure an index of contagion. There are several, but perhaps one of the more useful ones is the "k" of the negative binomial series.

3.4. Sampling to Overcome Problems of Microdistribution

Some understanding of "microdistribution" is useful for interpreting the sample data from ichthyoplankton surveys. "Microdistribution" is defined for this manual as the distributional characteristics of organisms near the scale of their body size or range of movement in a short period. This is distinguished from "hydrographic distribution" which is the distribution associated with particular ocean features such as eddies, upwelling areas, or islands. Both are distinguished from "physiological distribution" which is the entire geographic and bathymetric area in which the organism can survive. These three levels of distribution might be equated with the terms "aggregation," environmental preference," and "tolerance."

Ichthyoplankton survey precision is determined by the scale and intensity of microdistribution of the fish eggs and larvae being sampled. These two factors are of primary importance in estimating the number of samples required for a unit precision of spawning biomass estimate.

Fish eggs and larvae, as Cassie (1968) noted, " . . . are among the more variable in their spatial distribution, probably because they are relatively short-lived members of the plankton, and are initially released in a high over-dispersed (patchy) pattern." In addition, there appears to be a great difference between the dispersal of the spawning products of a demersal fish, the plaice (J.W. Talbot, Lowestoft, U.K., personal communication) and a pelagic schooling fish, the sardine (Smith, P.E., 1973). It may be that the plaice, in a relatively fixed position, discharges its eggs into moving water, while the sardine drifts along with the water and spawns into a rather persistent patch.

Presumably patch sizes will range from the area of spawning of a single female, through the entire area of spawning activity associated with large scale hydrographic features. Thus, the spawning activity is the origin of the distribution pattern of eggs, but subsequent events may modify that pattern. The spawning products may be transported away from the spawning area (Hirano and Fujimoto, 1968), they may disperse from patch centers (Smith, P.E., 1973), and local or wide-scale water motions may concentrate them (Fig. 3.27) in convergences (Langmuir, 1938; Owen, 1966) or gyrals. Dispersal may also result in open spaces between eggs that are large enough for samplers to pass through even though they are inside a "patch". Spaces may also be created by localized sources of mortality such as starvation or predation.

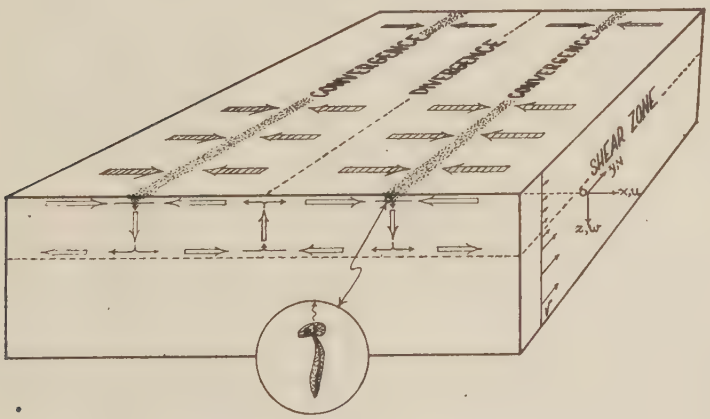


Figure 3.27. An example of a pattern-forming feature of the surface layer of the ocean which may interact with organism behavior to yield "strand lines" at convergences and areas of few organisms at divergences (Owen, 1966).

As yet there is no reliable way to measure the scale and intensity of pattern in the pelagic environment. Patch size can seldom be determined directly from the spawning products because they are not usually subject to direct observation or continuous measure-

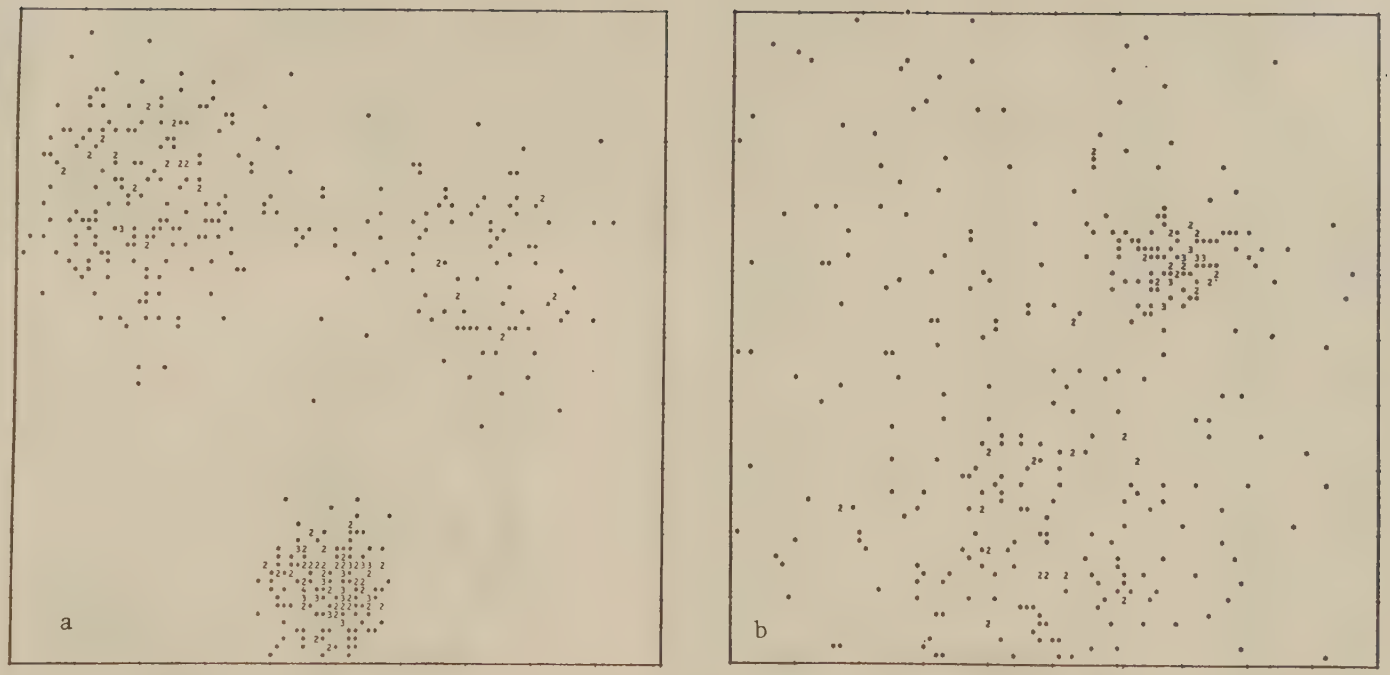


Figure 3.28. Computer simulated examples of centric aggregations. The mean concentration in (a) is 4.91 and the sample variance is 99.23. The mean concentration in (b) is 3.76 and the sample variance is 27.34. For comparison with the pattern in fish spawning, the "a" centric aggregation has an average negative binomial "k" of 0.256 and the "b" centric aggregation has a "k" of 0.600.

ment. Two indirect methods have provided a sense of scale of pattern. The first method, involving dispersal rate, was applied to sardine eggs by P.E. Smith (1973). The second method, involving sample size, has been borrowed from botanical techniques (Grieg-Smith, 1952; Pielou, 1969). Pattern has been described statistically by reference to the ratio of the variance to the mean (s^2/m) or some derivative of it such as the commonly used coefficient of variation (100 s/m). Lloyd's (1967) index of "mean crowding" also describes pattern and shows promise of distinguishing between various sources of mortality. Colebrook (1969) has shown that variance due to density gradients may be confused with patchiness.

Statistically, the best way, at present, to deal with the problems of microdistribution "is to increase the number of samples using the relationship S.E. = $S/\sqrt{n}$ where 'S.E.' is the standard error (standard error of the mean), and 'n' is the number of samples" (Cassie, 1968). An example is provided by the two computer simulated centric aggregations shown in Figure 3.28. These two distribution patterns differ in intensity but not in scale. For example, the above equation recommends that 411 samples be taken in order to get 10% accuracy in the mean concentration of the pattern in Figure 3.28 a. Only 193 samples need to be taken for the same precision in sampling the population in Fig. 3.28 b. The aggregation in Fig. 3.28 a has a mean density of 4.91, the sample variance is 99.23, and the average negative binomial "k" is 0.256. The aggregation in Fig. 3.28 b has a mean density of 3.76, the sample variance is 27.34 and "k" is 0.600.

3.5. Residence Time (Estimating Mortality)

One of the more difficult biases to measure in natural populations of eggs and larvae is "residence time" or period and extent of vulnerability to sampling. There are two simple techniques for measuring mortality which we will call the "cohort birth" method and the "constant birth" method. In the "cohort birth" method, the spawning products are released during a period of time which is short relative to the period of observation. The cohort (which refers to the group of eggs that is released at one time and whatever becomes of it) is then sampled in close successive time periods, and the mortality rate is estimated between each successive measurement. In the "constant birth" method, a single sample is analyzed for the abundance of a series of developmental stages of known age. It is beyond the scope of this manual to review all the necessary factors that effect these estimates such as drift of a cohort away from its point of origin (Smith, 1972), changes in the pattern and rate of dispersal (Smith, P.E. 1973), changes in the vulnerability of the larvae to sampling by day, night and size (Barkley, 1972; Lenarz, 1972, 1973; Murphy and Clutter, 1972) and the constancy of mortality rates among developmental stages (Fager, 1973). We will consider only the temperature-dependent hatching time (Ahlstrom, 1943; Lasker, 1964), the temperature-dependent larval growth rate (Lasker, 1964), and the food-dependent larval growth rate.

3.5.1. Temperature-Dependent Hatching Time

For annual estimates of spawning biomass, it may be necessary to make corrections for the temperature-specific hatching time because warm years speed the rate of development, reduce the residence times of the eggs, and cause an underestimate of the spawning population. Similarly, a colder-than-average year will cause the eggs to develop and hatch more slowly, thus increasing the length of time that eggs are vulnerable to sampling and causing an overestimate of the spawning biomass. The hatching time at various temperatures can be used to adjust the sample values to a central temperature and thereby correct the bias. The following table was developed from data provided by Lasker (1964) for Pacific sardine:

Temperature (j)	Hours to Hatch (t)	Resident Time Ratio (r)
13°	93.0	.65
14	78.5	.77
15	68.1	.88
16	60.2	1.00
17	53.7	1.12
18	48.4	1.24
19	42.2	1.39

Table 3.17. An example of the sample frequency distribution of Pacific sardine eggs and larvae.

CLASS BOUNDARY		EGGS			LARVAE (mm SL)						
LOWER	UPPER	A	B	C	3.00	4.75	5.75	6.75	7.75	8.75	9.75
1	4	7	14	5	13	5	12	23	21	32	23
4	16	14	14	16	36	20	32	51	52	19	12
16	64	20	27	31	50	46	68	45	18	8	2
64	256	36	33	17	29	49	30	7	1		
256	1024	22	25	26	23	34	3				
1024	4096	12	21	12	3	2					
4096	16384	6	5								
16384	65536	1									
	p	.645	.760	.591	.842	.852	.792	.689	.503	.322	.202
	n	118	139	107	154	156	145	126	92	59	37
	m	1044	734	385	153	189	54	22	13	8	6
	s	3465	1606	626	319	285	80	29	16	10	7
	k	.091	.209	.378	.230	.438	.457	.615	.648	.701	.715

In this instance, each sample value "x_{ij}" was multiplied by the residence time ratio "r_j" appropriate to each temperature "j" according to the following equation:

$$x_i = x_{ij}\ r_j$$

where x_{ij} = the number of eggs per 10 m^2 in the "i"th sample and its temperature "j", x_i is the 16°C - specific equivalent number of eggs per 10 m^2, r_j is the ratio t_{16}/t_j, t_{16} is the duration of the egg stage, from spawning to hatching, at 16°C, and t_j is the duration of the egg stage at temperature "j" in the same time units as t_{16}.

3.5.2. Temperature Dependent Larval Growth Rate

Larval growth is dependent on temperature as well as food. In sardine, the food used in early growth comes from the yolk, as opposed to feeding success. Thus, a table showing the effect of temperature on growth from hatching to the formation of a functional jaw can be made based on work by Lasker (1964):

Temperature	Hours to Jaw Formation	Residence Time Ratio
(j)	(t)	(r)
13°	121.0	0.60
14	101.5	0.78
15	81.9	9.89
16	72.8	1.00
17	63.3	1.15
18	55.6	1.31
19	49.8	1.46

The equation for calculating the 16°- equivalent abundance is the same as for hatching time (Section 3.5.1.). After formation of the jaw, the growth rate will likely

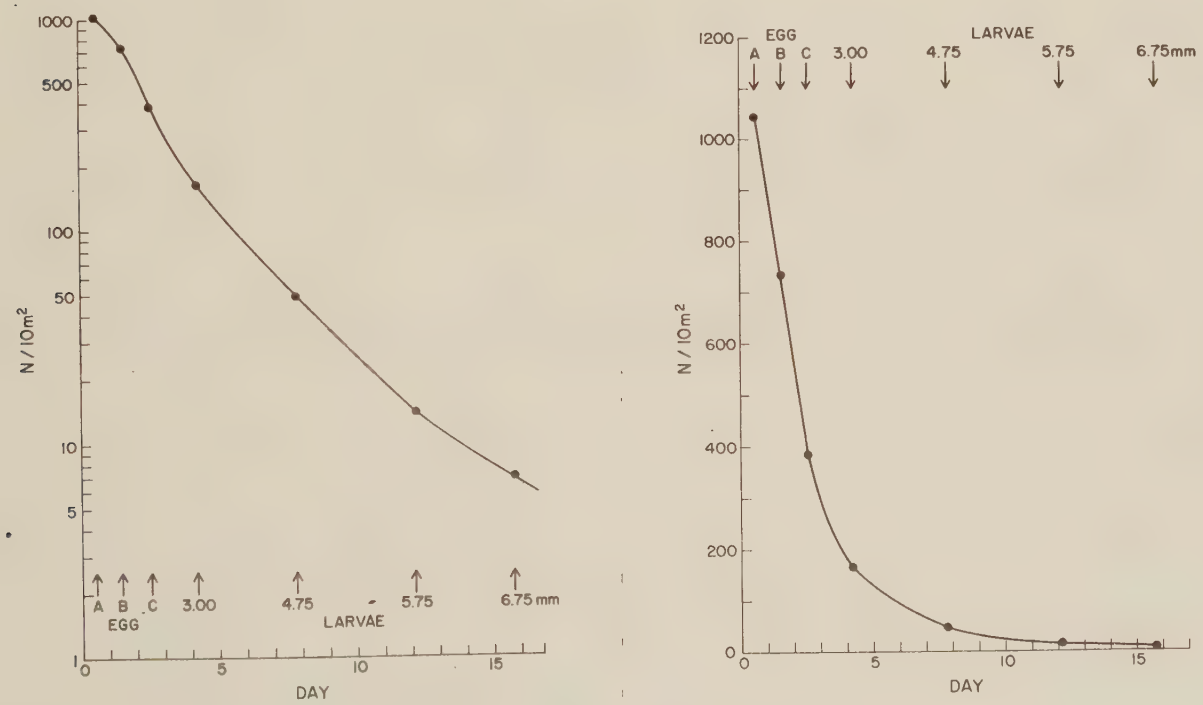


Figure 3.29. Apparent mortality rate of sardine eggs and larvae expressed on a logarithmic scale and an arithmetic scale (data from Ahlstrom, 1954).

be dependent on feeding success as well as temperature, which makes it more difficult to measure (Section 3.5.3.).

3.5.3. Food-Dependent Growth Rate

At present, there are no accurate methods for determining the food-dependent growth rate, nor for determining feeding-success in terms of abundance and composition of food particles in the field. "Cohort birth" methods of determining this value will be biased because of the probability that smaller members of the cohort, at the onset of feeding, will probably be more likely to starve than the larger larvae of the same cohort (Kramer and Zweifel, 1970). Also, the more active and larger larvae of a given cohort may attract more predation than the smaller more passive larvae.

3.5.4. Mortality Estimates

One attempt at deriving crude mortality rates was made by Ahlstrom (1954). These values illustrate one method of determining mortality rate:

	Eggs			Larvae			
	Early	Middle	Late	3.00	4.75	5.75	6.75mm
Individuals/10m^2	1044	734	385	395	234	56	22
Days at Stage	1	1	1	2.4	4.8	3.9	3.3
Individuals per day	1044	734	385	165	49	14	7
Age at mid-point	.5	1.5	2.5	4.2	7.8	12.15	15.75 days

Figure 3.29 illustrates this crude mortality rate on the logarithmic scale and the arithmetic scale. The data are derived from Table 3.17. At interpretation of an average line of this kind is that it represents "average" conditions relative to food and temperature. Future work on feeding and growth in the field may allow direct measurements of the food density and the interpretation of deviations from this crude mortality curve as explained by temperature and food density. Another bias was pointed out by Isaacs (1965). Since vulnerability to capture generally decreases rapidly in the feeding-larva stages, an explanation for increased catches of large larvae may be that those larvae which have starved become more vulnerable to capture. Further research is needed in the area of food-specific residence time, because growth may be arrested during starvation which leads to another unevaluated source of error.

3.6. Spawning Biomass Estimates

The fundamental relationship between the ichthyoplankton survey and the spawning biomass of a given fish stock is:

$$B = \frac{P}{C}$$

"B" is the spawning biomass of the fish stock, "C" is the census estimate of the egg production, and "P" is the capacity for production of eggs by a unit weight of fish stock. The production of eggs is determined by the fecundity of mature females expressed as eggs produced per unit time per unit weight and the proportion, by weight, of the mature stock made up of spawning females within each unit time. The appropriate census estimate of egg production is obtained by knowing the mean abundance of eggs in the spawning area, the mean residence time of the eggs in the water column, and the frequency of the egg census cruises.

The factors on which direct estimations of spawning biomass depend are not yet well enough known for most stocks to allow estimates of spawning biomass from ichthyoplankton surveys. The establishment of crude trial values for each parameter of fecundity and the subsequent refinement of these values to useful levels of accuracy and known levels of precision should be among the primary goals of ichthyoplankton research. Most of this manual is directed toward obtaining field estimates of egg production and analogous larva data from ichthyoplankton surveys. The procedures for determining population fecundity are not as well known and include the questions:

1) How many eggs are spawned by mature females per unit weight per unit time?

2) What proportion of the mature females are spawning at any given time?

3) How frequently does the average female spawn?

4) Over what period of time does each mature female in the population spawn once?

5) What is the seasonal distribution of spawning?

6) What is the geographic distribution of spawning by season?

7) To what degree does spawning rate or absolute fecundity depend on environmental conditions such as amount and availability of food?

8) What is the type and extent of variance for the above factors?

Until answers are found to the questions above, there are ways of obtaining preliminary estimates. The simplest (Smith, 1972) is to assume that the larval index is proportional to the spawning biomass alone. The equation of proportionality is then established using an independent estimate of spawning biomass, such as obtained by an analysis of the catch from each year class over the time it is in the fishery (Murphy, 1966). This simple equation is then used to extend the spawning biomass estimates into periods when the entire year class has not yet been captured.

Another method for estimating spawning biomass, is to determine the fecundity of mature females per unit weight and to arbitrarily assign a reasonable number of spawnings per year (Smith, 1972). This has been the primary approach of the ichthyoplankton surveys for the Pacific sardine. Analogous methods have been attempted for the northern anchovy. The important advantage of these preliminary attempts at the assessment of fish stock size is that they may be done with a minimum of information. The basic expense of these estimates is high for the primary species, but the addition of new species to the regional census is relatively inexpensive. If the general relationship between the larval index, or egg census and spawning biomass is sufficiently robust, the method will continue to provide a suitable check on catch analysis and lend supportive data for acoustic and aerial surveys.

3.7. Fecundity

The primary linkage between quantitative estimates of spawning intensity and coverage and estimates of the size of the spawning stock is fecundity (Saville, 1964). For new spawning surveys on relatively unfished stocks, it appears to be necessary to begin three phases of fecundity study. Before any fecundity studies have been done, the variations in spawning intensity are interpreted as changes of spawning biomass, fecundity and sampling error with the latter two assumed to be small relative to biomass changes. The first phase of the study of fecundity for the purpose of setting realistic limits to spawning biomass estimates is to determine the number of "advanced eggs" per gram of spawning female (MacGregor, 1968). The second phase is to determine the probability of a female attaining spawning condition by month or season. A third phase might be to understand the variations in fecundity from year-to-year, particularly in the number of batches spawned per year or alternatively the mean time between adjacent batches during the spawning season.

An example of the use of an egg or a larva census estimate with an independent estimate of the spawning biomass of a species was illustrated in Section 2. The time series of egg or larva census estimates may also be used alone to indicate time series trends in the relative spawning stock size. Estimates of absolute spawning stock biomass may be obtained if data exist on the egg production rate of females over a well-defined period (for example, a month or a year), the proportion of total spawning biomass which is male, the mortality rate of the eggs and larvae, and egg or larva census estimates which extend beyond the spawning region through the time of spawning.

Much of the fecundity information may be obtained from the catch through market sampling, from the incidental catch through sampling "trash fish" at sea, and similarly from exploratory surveys. The number of eggs per spawning per gram of female can usually be determined satisfactorily from a small sample (MacGregor, 1968). The

biomass sex ratio can similarly be estimated from market or sea survey samples. MacGregor (1968) found the mean and standard error for 19 female anchovies was 574 ± 10% (95% fiducial limits) eggs per gram of spawning female. This would translate to 5.74×10^8 eggs per metric ton of female biomass per spawning. If the male biomass is 75% of the female biomass, the population fecundity rate should be the same number of eggs per 1.75 metric ton of adult, or 3.28×10^8 eggs per metric ton of adult per spawning. If the adults may be expected to spawn two to three times per year, the eggs ($X=2\frac{1}{2}$) would be 8.00×10^8 eggs per metric ton of adult per year.

Smith (1972) compared three hypothetical models of anchovy spawning to check the sensitivity of spawning biomass estimates to those uncertainties about the variability in spawning behavior in the context of the increase in spawning biomass of the northern anchovy. The first model postulated a stable relationship between the annual larval census and the spawning biomass. The second model postulated the single spawning of each female in the winter quarter and does not respond to subsequent variation in spawning in the remaining seasons. The third model postulated the single spawning of each female in a maximum quarter (winter or spring in this instance). There were no apparent differences in the estimation of spawning biomass but the annual figure was subject to the least variation and the "maximum" season was subject to the most variation. Thus, the annual figure was chosen as the most representative and stable.

In a similar evaluation of the Pacific saury (Smith, Ahlstrom and Casey, 1970), the bimonthly spawning maximum was chosen because of (1) clear shifts in the seasonality of spawning; (2) migration and spawning outside the survey area in some months; and (3) evidence from the transpacific stock of this species that the egg batches can be matured each two months. (Hatanaka, 1956)

The Pacific hake spawn only in the CalCOFI grid area in a sharp peak at the same time each year. Relative spawning biomass has been calculated by integration from December to April, cruises in January-February, and cruises in January, February and March. Relative to the natural fluctuation in the 1951-60 period, the errors of estimate from the less complete surveys appear to be satisfactory.

Advanced research on the fecundity-spawning season problem should be based on the spawning characteristics of the individual species or determined from ichthyoplankton surveys and inspection of adult gonads. The population and individual fecundity characteristics to be determined can be expected to vary by major fish group and by latitude. For example, more or less continual spawning might be expected in the tropics and highly seasonal spawning could be expected in boreal zones. If spawning is essentially continuous, the primary data to be considered is the egg production per gram of female per unit time. A single annual spawning survey would be sufficient for this spawning behavior. If each spawning female spawns a single batch of eggs in a well-defined season, then the biomass estimate is derived from a few surveys during the spawning season. The most complete surveys and data on individual fecundity and population spawning behavior are required when there is multiple spawning at varying times during the year and between years. The primary data required is the egg production per gram of female per batch of eggs and the minimum time between repetitive spawnings.

Fundamental research on spawning is required for the direct use of egg and larva surveys in the direct absolute estimate of spawning biomass of any given species. In many species, even indirect estimates of spawning biomass obtained from spawning surveys may be the best available for unfished or underutilized species.

3.8. Alternative Quantitative Ichthyoplankton Samplers

The "slow" Bongo net system is recognized here as the best type of sampler for use in quantitative ichthyoplankton surveys. However, other systems may be used and produce acceptable results, provided they are used in a rigorously specified routine. In many instances, results are comparable to those obtained by Bongos (Section 3.9.).

Some of these alternative systems have been in use for many years. Thus, it

might be advisable to continue using an alternative sampling system, particularly if sampling performance has been well-documented over a long time period, until transfer to the standard equipment seems clearly advantageous. Also, there may be legitimate concern that the full-scale sampling system recommended here would be beyond the scope of the beginning ichthyoplankton program.

The major source of imprecision for each sampler will be the microdistribution pattern (Section 3.4.) of the eggs and larvae. Differences between samplers will primarily be related to volumetric sampling (Section 3.2.), i.e., volume and depth distribution of water filtered, the rates of avoidance in day and night tows, and the rates of escapement and extrusion through the meshes.

This section is not intended to describe these alternative samplers. (References are provided in the text.) Rather, the purpose is to review advantages and disadvantages of the major types of alternative sampling systems, relative to the Bongos, which may be used in quantitative ichthyoplankton surveys.

3.8.1. Oblique Tow Nets with Bridles

Many 1.0 m and 0.5 m nets have been designed and many are in use today. Descriptions of some of these are provided by Tranter and Smith (1968). One long-used example of this type of net is the CalCOFI net (Kramer et al, 1972) which consists of a cylindrical-conical net attached to a one-meter ring.

The chief advantage of this type of net is the simple and inexpensive bridle and ring used to launch and retrieve the net from the water. This process can even be done by hand, if necessary.

The main disadvantage, relative to the Bongo net, is the bridle preceding the net which causes directed water disturbances and results in increased avoidance. Problems associated with various types of materials used for the netting are discussed by Heron, 1968.

3.8.2. Vertical Tow Nets with Bridles

The first and most commonly used net which was designed to be towed vertically was the Hensen Egg net (Hensen, 1895; Simpson 1959, Santander and de Castillo, 1969).

Bridled 1.0m and 0.5m nets also fit into this category when hauled vertically. This type of sampling system is generally used in shallow depths.

The main advantage of vertical tow nets are: (1) simplicity in construction and (2) ease with which tow results may be converted to numbers of organisms per unit area of sea surface.

The chief disadvantage is the common practical limitation of equal sampling per unit depth. Other disadvantages include: (1) the sample is taken from water disturbed by the towing bridle all the way to the ship, thus avoidance may be great; (2) the rolling of the ship may alternately pull the net up rapidly and then let it fall, thus extruding organisms through the meshes on the ascent and expelling them from the mouth of the net on descent; (3) those nets having a small mouth restrict the size of the sample that can be taken.

3.8.3. High-Speed Nets

High-speed nets, designed to be towed faster than three knots, are still being developed and tested. Some standard forms have evolved (Fig. 3.30), e.g., the Gulf III (Gehringer, 1952, 1962) and modifications of it (Bridger, 1958; Nellen and Hempel, 1969; Zijlstra, 1970; Harding and Arnold, 1971). The newer high-speed nets appear to be satisfactory for estimating spawning biomass (Bjorke, Dragesund, and Ulltang, 1974; Gjosaeter and Saetre, 1974; Postuma and Zijlstra, 1974; Saville, Baxter, and McKay, 1974; Schnack, 1974).

One advantage of the high-speed type net is that it allows one to make headway to the next station during the tow, thereby saving ship time and money. Other

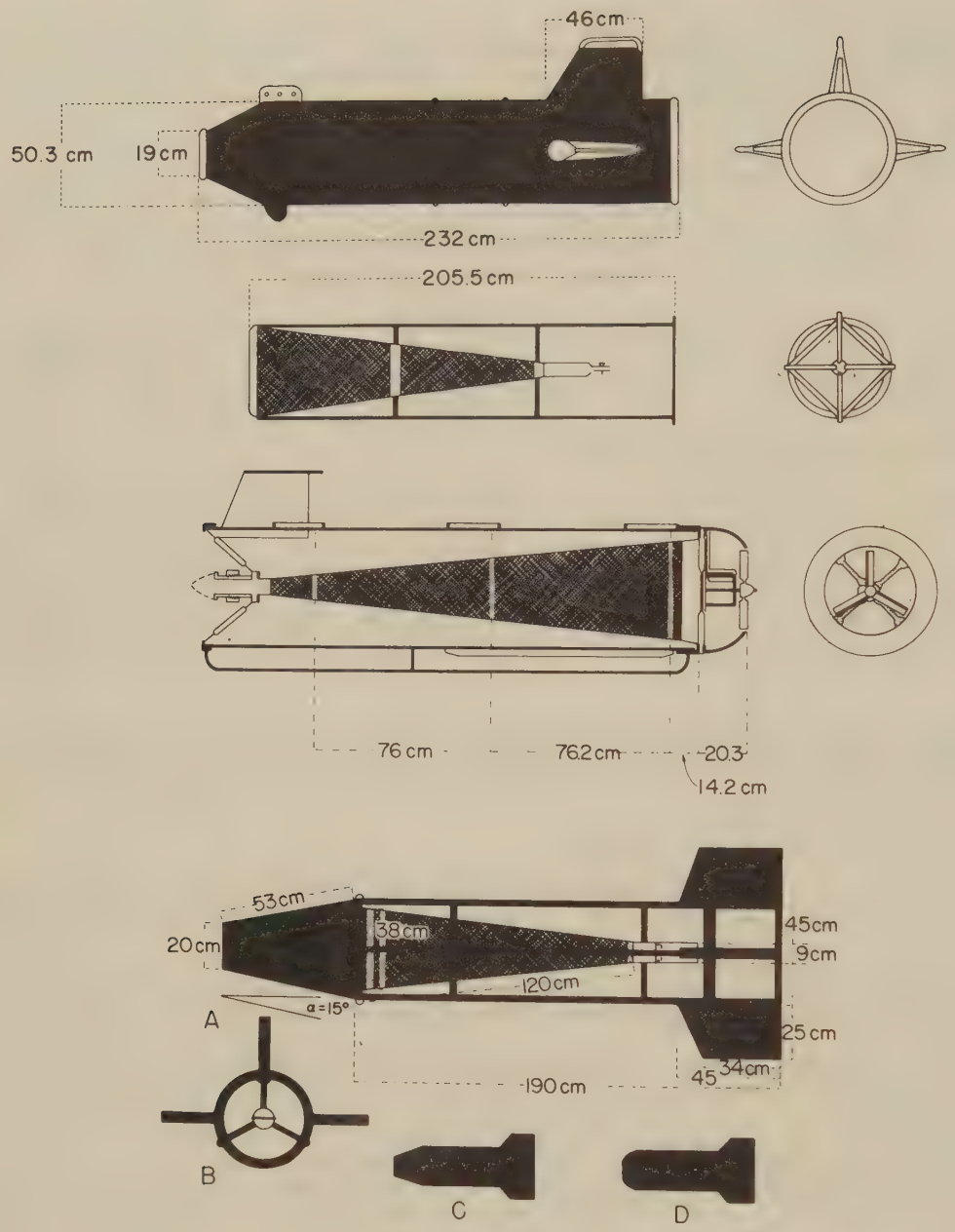


Figure 3.30. Scale drawings of three high-speed samplers used in the ICES (International Council for Exploration of the Sea) Larval Herring Survey Program in the North Sea. TOP) Dutch Gulf III (Gehringer, 1952; Bridger, 1958; Zijlstra, 1970) The net is of 0.4 mm nylon mesh and is suspended on the frame shown below the body of the sampler and the frame is inside the sampler body when under tow. MIDDLE) The Lowestoft High-Speed Plankton Sampler (Beverton and Tungate, 1967; Harding and Arnold, 1971) The net is of 0.3 mm nylon mesh. BOTTOM) German type high-speed net "Nackthai" (Nellen and Hempel, 1969) The net is of 0.3 mm nylon mesh. The net differs from the nets above by the absence of a casing surrounding the net. All three nets are fished at a towing speed of five knots and the double oblique tow to within five m of the bottom is accomplished by paying out and recovering the towing cable at ca. 20 meters per minute. The nets should have time depth recorders and flowmeters.

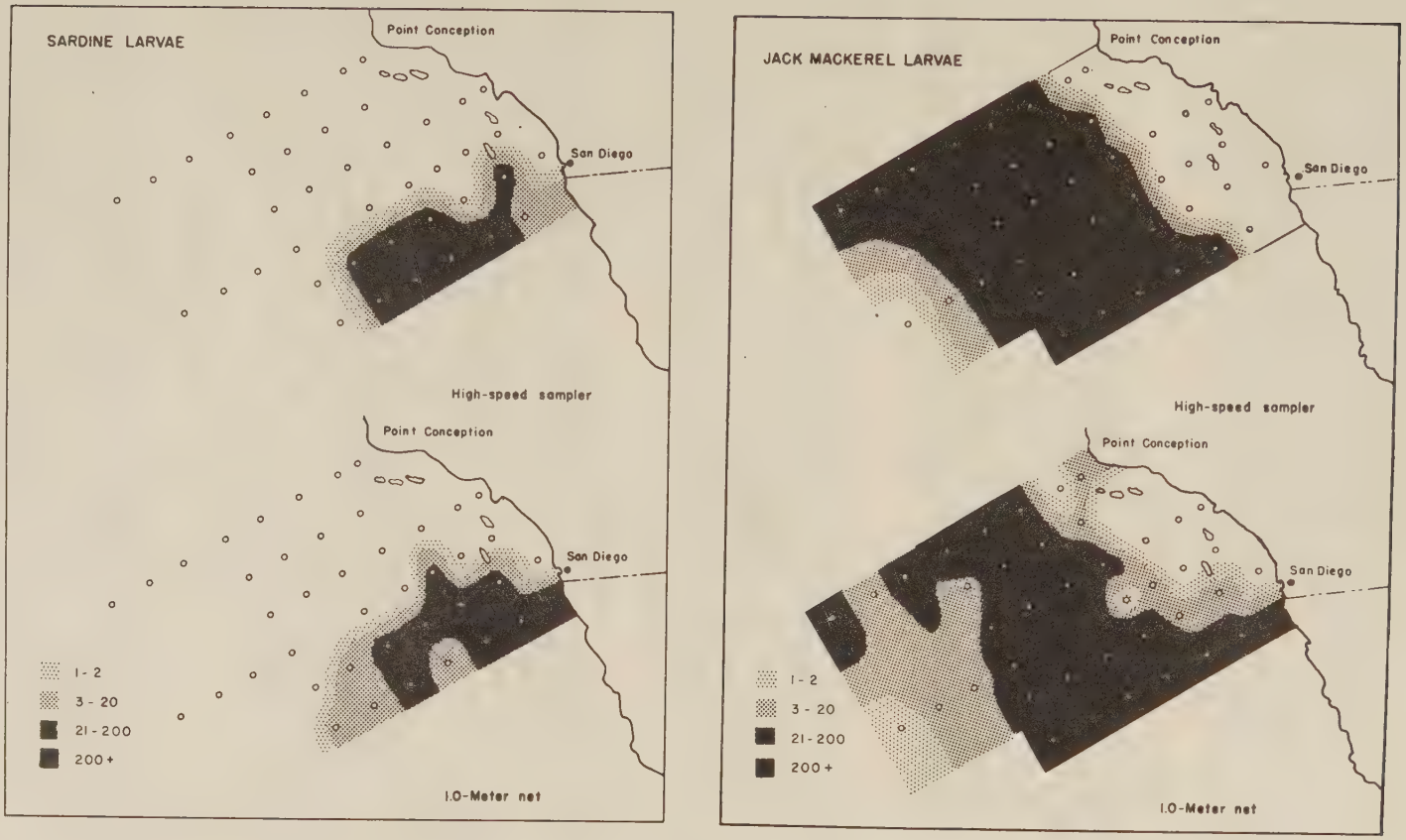


Figure 3.31. A direct comparison of results from two radically different ichthyoplankton samplers. Census estimates for sardine larvae were 477 x 10^9 for the CalCOFI one-meter net and 502 x 10^9 for the Isaacs high-speed sampler. Census estimates for jack mackerel larvae were 4.8 x 10^{12} for the CalCOFI one-meter net and 6.6 x 10^{12} for the Isaacs high-speed sampler. (Figure 9, page 198, and Figure 12, page 202 from Ahlstrom, Isaacs, Thrailkill and Kidd, 1958).

advantages include: (1) the speeds of tow are frequently high enough to allow sea current and wind conditions to be ignored and thus a rigorously controlled tow program is possible under severe sampling conditions; (2) larger specimens of the species are caught allowing mortality rates to be calculated for a larger portion of their planktonic life.

Disadvantages include: (1) filtration pressure is often high and variable which causes damage to and loss of smaller larvae; (2) the high speed requires a varying amount of cable for each increment of depth and this may make a straight tow path difficult, which could result in oversampling the surface waters; (3) the initial cost of the equipment and the more complicated methods of launch and recovery of the nets may be prohibitive for some ships and programs; (4) the small mouth sizes may not allow enough water to be filtered to pick up the larger rarer organisms which high-speed samplers are intended to catch.

3.9. Intercalibration of Quantitative Samplers

One may wish to intercalibrate samplers to allow comparison of (1) older data with that currently being collected, (2) cooperative surveys using different samplers, and (3) samplers which collect some overlapping size categories of ichthyoplankton. The standards of comparison should not differ from the statistical standards one applies to the survey results, therefore the estimation of the number of samples required can be derived from Table 3.6 (p. 52). The number of 'replicate' samples should be calculated from a trial series of samples with both samplers or may be estimated from the variance in the existing data from one of the samplers.

The intercalibration must account for all of the major sources of error: avoidance, escapement, extrusion, biased sampling of the water column, and different scales of sample size. Samplers should be compared under conditions which allow the comparison of the mean and variance per unit surface area and particularly the ability to detect the boundaries of the geographic distribution. For fish larvae, a comparison of the size composition of the catch is critical. It is unlikely that sample formats which change the scale of sampling radically will yield the same sample variance in comparisons and the services of a trained statistician should be sought for further tests of this phenomenon.

The complete results of a comparison of a high speed sampler with a 2.5 cm opening, towed in a horizontal series at 10, 20, 30, and 40 meters, and an oblique tow with a one-meter ring net towed from 70 meters to the surface is available in Ahlstrom, et al (1958). In this test, conducted over a 72,000 square nautical mile area in May of 1950, comparisons were made of total plankton volume, sardine eggs and larvae, anchovy larvae, jack-mackerel larvae, lanternfishes and other fish larvae. The horizontal distribution maps from this study are shown in Figure 3.31. From these comparisons one can see that the centers of abundance and distributional boundaries were well-represented by both nets. The high-speed net was ultimately rejected because it did not catch sufficient numbers of large larvae in its 40 m^3 sample relative to those caught by the larger slower net with a sample 10 times as large, and because the high-speed towing apparatus at that time was not capable of being fished to the depth required to sample under the distributions of larvae in this region. Nonetheless, the results from this comparison can be used to design adequate comparisons of standard sampler performance.

3.10. Design of Surveys to Minimize Costs

Table 3.18 lists the various cost elements of the CalCOFI surveys. Obviously, the increased efficiency of surveys must depend on reducing the major costs without substantially reducing the precision. Cursory inspection indicates that the greatest cost elements are shiptime, administration and technical personnel. Basic reductions in ship's costs can be made by: (1) reducing the number of cruises per year, (2) reducing the number of lines per cruise, and (3) reducing the number of stations per line. The recommended procedure is to conduct thorough cruise schedules in the exploratory and analytical phases of the egg and larva survey, then use the data from these phases to design the most effective and efficient monitoring cruises. It is possible that complete surveys need to be made only each two or three years with supplementary data being taken in the interim years.

Also, it may be convenient or necessary to merge sea operations of basic oceanography, exploratory fishing, acoustic surveys, pollution monitoring, or data buoy tending with the operations of egg and larva surveys. This can result in considerable savings in the sea-going phase of the egg and larva survey or alternately may provide extra samples with which to augment the standard survey. One must take precautions that the adventitious sampling program is an unbiased and representative set of samples. For example, one might need to adjust data from groups of samples taken only at night to compare with samples taken at all times of a 24-hour day. Samples taken over a small area may not be representative of a large area. Provided the sampling characteristics of the joint surveys are mutually satisfactory to the cooperators, massive savings are possible. For example, the CalCOFI surveys have profitted from this joint planning and conduct, and the potential now exists for the extensive environmental data to be used with the larval survey data to design experiments to test the relationships among stock size, larval survival, juvenile survival and recruitment.

Table 3.18. Estimated cost of a decade of ichthyoplankton surveys conducted over 300,000 square nautical miles of temperate, eastern boundary current off the west coast North America (CalCOFI 1972-base dollars).

CATEGORIES	PERSONNEL	DAYS/YEAR	$/DAY	YEARS	
DIRECT SURVEY COSTS					
Biological Aids	9	240	25	10	$ 540,000
Biological Technicians	34	240	50	10	4,080,000
Fishery Biologist	1	240	100	10	240,000
50 m Research Vessel	2	200	2500	10	10,000,000
SURVEY INTERPRETATION					
Fecundity Estimate					
Biological Technician	1	240	50	2	24,000
Fishery Biologist	1	240	100	2	48,000
Temperature Specific Hatching and Development Times					
Biological Technician	1	240	50	3	36,000
Fishery Biologist	1	240	100	3	72,000
Data Processing and Statistical Analysis					
Fishery Biologist	1	240	100	4	96,000
Biological Technician	1	240	50	4	48,000
Computer Time		240	2	4	1,920
Decade Total					15,185,920
Administrative Overhead (50%)					7,592,960
TOTAL					22,778,880

4. REFERENCES

Ahlstrom, E.H., 1943 Studies on the Pacific pilchard or sardine (Sardinops caerulea). 4. Influence of temperature on the rate of development of pilchard eggs in nature. Spec.Sci.Rep.USFWS, (23):26 p.

_____, 1954 Distribution and abundance of egg and larval populations of the Pacific sardine. Fish.Bull.USFWS, 56:82-140

_____, 1959 Vertical distribution of pelagic fish eggs and larvae off California and Baja California. Fish.Bull.USFWS, 60:106-46

_____, 1967 Co-occurrences of sardine and anchovy larvae in the California Current region off California and Baja California. Rep.CCOFI, (11):117-35

_____, 1968 An evaluation of the fishery resources available to California fishermen. Univ.Wash.Publ.Fish.(New Ser.), (4):65-80

_____ (ed.), 1969 Recommended procedures for measuring the productivity of plankton standing stock and related oceanic properties. 5. Sampling zooplankton to determine biomass. Washington, D.C., National Academy of Sciences, pp.47-59

_____, 1971 Kinds and abundance of fish larvae in the eastern tropical Pacific, based on collections made on EASTROPAC I. Fish.Bull.NOAA/NMFS, 69(1): 3-77

_____, 1972 Kinds and abundance of fish larvae in the eastern tropical Pacific on the second multi-vessel EASTROPAC survey, and observations on the annual cycle of larvae abundance. Fish.Bull.NOAA/NMFS, 70(4):1153-242

Ahlstrom, E.H. and J.R. Thrailkill, 1963 Plankton volume loss with time of preservation. Rep.CCOFI, (9):57-73

Ahlstrom, E.H. et al., 1958 High speed plankton sampler. Fish.Bull.USFWS, 58:187-214

Ahlstrom, E.H. et al., 1969 Sampling zooplankton to determine biomass. In Recommended procedures for measuring the productivity of plankton standing stock and related oceanic properties, edited by E.H. Ahlstrom. Washington, D.C., National Academy of Sciences, 59 p.?

Aitchison, J. and J.A.C. Brown, 1966 The Pognormal distribution. Cambridge, University Press

Anscombe, F.J., 1949 The statistical analysis of insect counts based on the negative binomial distribution. Biometrics, 5:165-73

Bagenal, M., 1955 A note on the relations of certain parameters following a logarithmic transformation. J.Mar.Biol.Assoc.U.K., 34:289-96

Barkley, R.A., 1964 The theoretical effectiveness of towed-net samplers as related to sampler size and the swimming speed of organisms. J.Cons.CIEM, 29(2): 146-57

_____, 1972 Selectivity of towed net samplers. Fish.Bull.NOAA/NMFS, 70:799-820

Beverton, R.J.H. and S.J. Holt, 1957 On the dynamics of exploited fish populations. Fish.Invest.Minist.Agric.Fish.Food G.B. (2 Sea Fish.), (19):533 p.

Beverton, R.J.H. and D.S. Tungate, 1967 A multi-purpose plankton sampler. J.Cons.CIEM, 31(2):145-57

Birge, E.A., 1895 Plankton studies on Lake Mendota. 1. The vertical distribution of the pelagic crustacea during July, 1894. Trans.Wis.Acad.Sci.Arts Lett., 10: 421-84

Bjorke, H., O. Dragesund and Ø. Ulltang, 1974 Efficiency test on four high-speed plankton samplers. In The early life history of fish, edited by J.H.S. Blaxter. Berlin, Springer-Verlag, pp.183-200

Bridger, J.P., 1958 On efficiency tests made with a modified Gulf III high-speed tow-net. J.Cons.CIEM, 23:357-65

Cassie, R.M., 1968 Sample design. Unesco Monogr.Oceanogr.Methodol., (2):105-21

Clutter, R.I., 1969 The macrodistribution and social behaviour of some pelagic mysid shrimps. J.Exp.Mar.Biol.Ecol., 3:125-55

Clutter, R.I. and M. Anraku, 1968 Avoidance of samplers. Unesco Monogr.Oceanogr.Methodol., (2):57-76

Colebrook, J.M., 1969 Variability in the plankton. Progr.Oceanogr., 5:115-25

_____, 1973 The design of sampling surveys. FAO.Fish.Tech.Pap., (122):52-8

Cushing, D.H. and J.G.K. Harris, 1973 Stock and recruitment and the problem of density-dependence. Rapp.P.-V.Réun.CIEM, 164:142-56

Davies, I.E. and E.G. Barham, 1969 The Tucker opening-closing mikronekton net and its performance in a study of the deep scattering layer. Mar.Biol., 2(2): 127-31

Ege, V., 1930 Sudidae (Paralepis). Rep.Dan.Oceanogr.Exped.Mediterr., 2A (13):193 p.

_____, 1953 Paralepididae. 2. (Macroparalepsis). Taxonomy, ontogeny, phylogeny and distribution. Dana Rep., (40):184 p.

_____, 1957 Paralepididae. 2. (Macroparalepsis). Taxonomy, ontogeny, phylogeny and distribution. Dana Rep., (43):101 p.

Fager, E.W., 1973 Estimation of mortality coefficients from field samples of zooplankton. Limnol.Oceanogr., 18(2):297-301

Fagetti, E., 1970 Distribution and relative abundance of Clupeidae and Engraulidae larvae in the waters of the continental shelf of Senegal and Gambia during 1969. Rapp.Sci.Provis.Cent.Rech.Oceanogr.Dakar-Thiaroye, (1/70):26 p.

Fisher, R.A., 1941 The negative binomial distribution. Ann.Eugen., 11:182-7

Forbes, S.T. and O. Nakken, 1972 Manual of methods for fisheries resources survey and appraisal. Part 2. The use of acoustic instruments for fish detection and abundance estimation. FAO Man.Fish.Sci., (5):138 p.

Fraser, J.H., 1969 Variability in the oceanic content of the plankton in the Scottish area. Progr.Oceanogr., 5:149-59

Gehringer, J.W., 1952 An all-metal plankton sampler (model Gulf III). Spec.Sci.Rep.USFWS (Fish.), (88):7-12

Gehringer, J.W., 1962 The Gulf III and other high-speed plankton samplers. Rapp.P.-V.Réun. CIEM, 153:19-22

Gjøsaeter, J. and R. Saetre, The use of data on eggs and larvae for estimating
1974 spawning stock of fish populations with demersal eggs. In The early life history of fish, edited by J.H.S. Blaxter. Berlin, Springer-Verlag, pp.139-49

Grieg-Smith, P., The use of random and contiguous quadrats in the study of structure
1952 of plant communities. Ann.Bot., 16:293-316

Gulland, J.A., Manual of sampling and statistical methods for fisheries biology.
1966 Part 1. Sampling methods. FAO Man.Fish.Sci., (3):87 p. (Repr.1971)

_____, Manual of methods for fish stock assessment. Part 1. Fish population
1969 analysis. FAO Man.Fish.Sci., (4):154 p.

_____ (comp.), The fish resources of the ocean. FAO Fish.Tech.Pap., (97):
1970 425 p.

Harding, P., and G.P. Arnold, Flume experiments on the hydro-dynamics of the Lowestoft
1971 high-speed plankton samplers. J.Cons.CIEM, 34(1):24-36

Hatanaka, M., Biological studies on the population of the saury, Cololabis saira
1956 (Brevoort). 1. Reproduction and growth. Tohuku J.Agric.Res., 6(3):227-69

Hayashi, S., Fishery biology of the Japanese anchovy, Engraulis japonica (Houttuyn).
1961 Bull.Tokai Reg.Fish.Res.Lab., (31):145-268

Hempel, G. (ed.) Fish eggs and larval studies. (Contributions to a manual).
1973 FAO Fish.Tech.Pap., (122):82 p.

Hempel, G. and H. Weikert, The neuston of the subtropical and boreal northeastern
1972 Atlantic Ocean: a review. Mar.Biol., 13(1):70-88

Hensen, V., Methodik der Untersuchungen bei Planktonexpedition. Ergebn.Atl.Ozean.
1895 Planktonexped.Humboldt-Stiffing, 1:200 p.

Hensen, V. and C. Apstein, Die Nordsee-Expedition 1895 des Deutschen See fischerei-
1897 Vereins. Uber die Eimenge der im Winter laichenden Fische. Wiss. Meeresunters.Helgoland, 2(2):101 p.

Heron, A.C., Plankton gauze. Unesco Monogr.Oceanogr.Methodol., (2):19-26
1968

Hirano, T. and M. Fujimoto, Preliminary results of investigations of the Kuroshio
1970 functioning as a means of transportation and diffusion of fish eggs and larvae. In The Kuroshio, a Symposium on the Japan Current, edited by J.C. Marr, Honolulu, East-West Center Press, pp.405-16

ICNAF, North Atlantic fish marking symposium, Woods Hole, Mass., May 1961.
1963 Spec.Publ.ICNAF, (4):370 p.

Isaacs, J.D., Night-caught and day-caught larvae of the California sardine.
1965 Rep.CCOFI, (10):102-40

Isaacs, J.D., A. Fleminger and J.K. Miller, Distributional atlas of zooplankton bio-
1969 mass in the California Current region : Spring and Fall, 1955-59. Atlas CCOFI, (10):252 p.

Josai, J.W., Annotated bibliography of zooplankton sampling devices. NOAA Tech.Rep.
1970 NMFS/SSRF, (609):90 p.

Klawe, W.L., Observations on the spawning of four species of tuna, Neothunnus macro-
1963 pterus, Katsuwonus pelamis, Auxis thazard and Euthynnus lineatus, in the eastern Pacific Ocean, based on the distribution of their larvae and juveniles. Bull.I-ATTC 6(9):448-540

Kofoid, C.A., 1897 On some important sources of error in the plankton method. Science, N.Y., 6(153):829-32

Kramer, D., 1963 Records and observations from plankton grid studies off Baja California, April 1952. Spec.Sci.Rep.USFWS(Fish.), (442):42 p.

Kramer, D. and J.R. Zweifel, 1970 Growth of anchovy larvae (Engraulis mordax Girard) in the laboratory as influenced by temperature. Rep.CCOFI, (14):84-7

Kramer, D. and P.E. Smith, 1971 Seasonal and geographic characteristics of fishery resources : California Current region. 5. Northern Anchovy. Commer.Fish. Rev., 33(3):33-8

Kramer, D. et al., 1972 Collecting and processing data on fish eggs and larvae in the California Current region. NOAA Tech.Rep.NMFS Circ., (370):38 p.

Langmuir, I., 1938 Surface motion of water induced by wind. Science,N.Y., 87:119-23

Lasker, R., 1964 An experimental study of the effect of temperature on the incubation time, development, and growth of Pacific sardine embryos and larvae. Copeia, 1964 (2):399-405

Lenarz, W.H., 1972 Mesh retention of larvae of Sardinops caerulea and Engraulis mordax by plankton nets. Fish.Bull.NOAA/NMFS, 70(3):839-48

_____, 1973 Dependence of catch rates on size of fish larvae. Rapp.P.-V.Réun. CIEM, 164:270-5

Lloyd, M., 1967 "Mean crowding". J.Anim.Ecol., 36:1-30

Longhurst, A.R., 1967 Vertical distribution of zooplankton in relation to the eastern Pacific oxygen minimum. Deep-Sea Res., 14:51-63

MacGregor, J.S., 1968 Fecundity of the Pacific sardine (Sardinops caerulea). Fish.Bull. USFWS, 57:427-49

Mackintosh, N.A., 1934 Distribution of the macroplankton in the Atlantic sector of the Antarctic. Discovery Rep., 9:65-160

Mahnken, C.V.W. and J. Jossi, 1967 Flume experiments on the hydrodynamics of plankton nets. J.Cons.CIEM, 31(1):38-45

Matsumoto, W.M. et al., 1972 On the clarification of larval tuna identification particularly in the genus Thunnus. Fish.Bull.NOAA/NMFS, 70(1):1-12

May, R.C., 1970 Feeding larval marine fishes in the laboratory : a review. Rep.CCOFI, (14):73-83

_____, 1971 Effects of delayed initial feeding on larvae of the grunion Leuresthes tenius (Ayres). Fish.Bull.NOAA/NMFS, 69(2):411-25

McEwen, G.F., M.W. Johnson and T.R. Folsom, 1954 A statistical analysis of the performance of the Folsom plankton sample splitter based upon test observations. Arch.Meteorol.Geophys.Bioklimatol. (A Meteorol.Geophys.), 7:502-27

McGowan, J.A. and D.M. Brown, 1966 A new opening-closing paired zooplankton net. SIO Ref.(Scripps Inst.Oceanogr.), (66-23)

Milne, A.A., 1959 The centric systematic area-sample treated as a random sample. Biometrics, 15(2):270-97

Moser, H.G., and E.H. Ahlstrom, 1970 Development of lanternfishes (family Myctophidae) in the California Current. Part 1. Species with narrow-eyed larvae. Bull.Los Ang.Cty.Mus.Nat.Hist.Sci., (7):145 p.

Moser, H.G. and E.H. Ahlstrom, Development of the lanternfish (Scopelopsis multi-
1972 punctatus Brauer 1906), with a discussion of its phylogenetic position in the family Myctophidae and its role in the proposed mechanism for the evolution of photophore patterns in lanternfishes. Fish.Bull.NOAA/NMFS, 70(3):541-64

Motoda, S. et al., Proposed method of estimation of quantity of water filtered by
1963 vertical net haul, and its application on illustrated distribution of zooplankton biomass in the eastern Indian Ocean. Inf.Bull.Planktol.Japan, (10):22-8

Murphy, G.I., Population biology of the Pacific sardine (Sardinops caerulea). Proc.
1966 Calif.Acad.Sci., 34:1-84

Murphy, G.I. and R.I. Clutter, Sampling anchovy larvae with a plankton purse seine.
1972 Fish.Bull.NOAA/NMFS, 70:789-98

Nellen, W. and G. Hempel, Versuche zur Fängigkeit des "Hai" und des modifizierten
1969 Gulf-V-Plankton-Samplers "Nackthai". Ber.Dtsch.Komm.Meeresforsch., 20(2):141-54

Nellen, W. and R.C. Newell, Marine plankton : a practical guide. London, Hutchinson
1963 Educational, 207 p.

Owen, R.W. Jr., Small-scale horizontal vortices in the surface layer of the sea.
1966 J.Mar.Res., 24(1):56-66

Parin, N.V., Ichthyofauna of the epipelagic zone. Moskva, Izdatel'-stov Nauka,
1968 186 p. Issued also in English by Israel Program for Scientific Translations. Jerusalem, IPST Cat.No.5528:210 p. (1970)

Parrish, R.H., Exploitation and recruitment of Pacific mackerel, Scomber japonicus
1974 in the north eastern Pacific. Rep.CCOFI, (17):136-40

Pertseva-Ostroumova, T.A., Some morphological characteristics of myctophid larvae
1964 (Myctophidae, Pisces). In Fishes of the Pacific and Indian Oceans, biology and distribution, edited by T.S. Ross. Tr.Inst.Okeanol., 73: 79-97 (in Russian)

Pielou, E.C., An introduction to mathematical ecology. New York, Wiley, 286 p.
1969

Posgay, J.A., R.R. Marak and R.C. Hennemuth, Development and test of new zooplankton
1968 samplers. ICNAF Res.Doc. (85)(mimeo)

Postuma, K.H. and J.J. Zijlstra, Larval abundance in relation to stock size,
1974 spawning potential and recruitment in North Sea herring. In The early life history of fish, edited by J.H.S. Blaxter. Berlin, Springer-Verlag, pp.113-28

Reighard, J., Methods of plankton investigation in their relation to practical
1898 problems. Bull.U.S.Fish.Comm., (17):169-75

Richards, W.J. and D.C. Simmons, Distribution of tuna larvae (Pisces, Scombridae)
1971 in the Northwestern Gulf of Guinea and off Sierra Leone. Fish.Bull.USFWS, 69(3):555-68

Sameoto, D.D. and L.O. Jaroszynski, Other surface samples : a new neuston net.
1969 J.Fish.Res.Board Can., 26(8):2240-4

Santander, H. and O.S. de Castillo, El desove de la anchoveta (Engraulis ringens J.)
1969 en los periodos reproductivos de 1961 a 1968. Inf.Espec.Inst.Mar Peru, (40):1-10

Saville, A., Mesh selection in plankton nets. J.Cons.CIEM, 23:192-20
1959

Saville, A., I.G. Baxter and D.W. McKay, Relations between egg production, larval
1974 production and spawning stock size in Clyde herring. In The early life history of fish, edited by J.H.S. Blaxter. Berlin, Springer-Verlag, pp.129-38

Schaeffer, M.B., A study of the dynamics of the fishery for yellowfin tuna in the
1957 eastern tropical Pacific Ocean. Bull.I-ATTC, 2(6):247-85

Schnack, D., On the reliability of methods for quantitative surveys of fish larvae.
1974 In The early life history of fish, edited by J.H.S. Blaxter. Berlin, Springer-Verlag, pp.201-12

Sette, O.E. and E.H. Ahlstrom, Estimations of abundance of the eggs of the Pacific
1948 pilchard (Sardinops caerulea) off southern California during 1940 and 1941. J.Mar.Res., 7(3):511-42

Silliman, R.P., Analog computer models of fish populations. Fish.Bull.USFWS, 66(1):
1967 31-46

Simpson, A.C., The spawning of plaice in the North Sea. Fish.Invest.Minist.Agric.
1959 Fish.Food G.B. (2 Sea Fish.), 22(7):111 p.

Smith, P.E., The increase in spawning biomass of northern anchovy Engraulis mordax.
1972 Fish.Bull.NOAA/NMFS, 70(3):849-74

_____, The mortality and dispersal of sardine eggs and larvae. Rapp.P.-V.
1973 Réun.CIEM, 164:282-92

Smith, P.E. and R.I. Clutter, Hydrodynamics of flow and collection in plankton nets.
1965 In Ocean science and ocean engineering. Transactions of the Joint conference and exhibit, 14-17 June 1965, sponsored by the Marine Technology Society and the American Society of Limnology and Oceanography. Washington, D.C., Marine Technology Society/ASLO, vol.1:515 (Abstr.)

Smith, P.E., E.H. Ahlstrom and H.D. Casey, The saury as a latent resource of the
1970 California Current. Rep.CCOFI, (14):88-130

Smith, P.E., R.C. Counts and R.I. Clutter, Changes in filtering efficiency of
1968 plankton nets due to clogging under tow. J.Cons.CIEM, 32(2):232-48

Smith, P.E., J.R. Thrailkill and A.N. Vrooman, Cruise report, J-59. La Jolla,
1971 California, Southwest Fisheries Center, NOAA/NMFS (Unpubl.)

Southwood, T.R.E., Ecological methods. London, Methuen, 391 p.
1966

Squire, J.L., Jr., Apparent abundance of some pelagic marine fishes off the southern
1972 and central California coast as surveyed by an airborne monitoring program. Fish.Bull.NOAA/NMFS, 70(3):1005-20

Steedman, H.R. (ed.), Zooplankton fixation and preservation. Unesco Monogr.Oceanogr.
1976 Methodol., (4):350 p.

Sund, P.N. and W.J. Richards, Results of trials with neuston net in the Gulf of
1965 Guinea. In Ocean science and ocean engineering, Transactions of a Joint conference and exhibit, 14-17 June 1965 sponsored by the Marine Technology Society and the American Society of Limnology and Oceanography. Washington, D.C., Marine Technology Society/ASLO, vol.1:515-23

Switzer, P., Reconstructing patterns from sample data. Ann.Math.Stat., 38:138-54
1967

Tate, N.W. and R.C. Clelland, Non-parametric and short-cut statistics. Danville,
1957 Ill., Interstate Publishers

Taylor, C.C., 1953 Nature of variability in trawl catches. Fish.Bull.NOAA/NMFS, 54: 145-66

Tranter, D.J. (ed.), 1968 Zooplankton sampling. Unesco Monogr.Oceanogr.Methodol., (2): 174 p.

Tranter, D.J. and P.E. Smith, 1968 Filtration performance. Unesco Monogr.Oceanogr. Methodol., (2):27-56

Vannucci, M., 1968 Loss of organisms through the meshes. Unesco Monogr.Oceanogr.Methodol., (2):77-86

Vrooman, A.M., 1972 Cruise report. La Jolla, California, Southwest Fisheries Center, NOAA/NMFS (Unpubl.)

Vrooman, A.M. and P.E. Smith, 1972 Biomass of the subpopulation of northern anchovy (Engraulis mordax Girard), Rep.CCOFI, (15):49-51

Zaitsev, Yu.P., 1970 Marine neustonology. Kiev, Naukov Dumka, 264 p. (in Russian)

Zijlstra, J.J., 1970 Herring larvae in the central North Sea. Ber.Dtsch.Wiss.Komm. Meeresforsch., 21:92-115

Zweifel, J.R., 1973 A non-parametric approach to the estimation of relative change in fish population size from egg and larval surveys. Rapp.P.-V.Réun.CIEM, 164: 276-81

M-43
ISBN 92-5-100515-X